ANNUAL PROGRESS REPORT

RESEARCH ON NAVY-RELATED COMBAT CASUALTY CARE ISSUES, NAVY OPERATIONAL-RELATED INJURIES AND ILLNESSES AND APPROACHES TO ENHANCE NAVY/MARINE CORPS PERSONNEL COMBAT PERFORMANCE

Prepared for

Naval Medical Research Institute
Bethesda, Maryland 20814

As Required By
Contract Number N00014-95-D-0048
(GC-2728)

Prepared by
GEO-CENTERS, INC.
7 Wells Avenue
Newton Centre, MA 02159

January 1996

GEO-CENTERS, INC.

TABLE OF CONTENTS

ANNUAL PROGRESS REPORT
INCLUDING PROGRESS FOR 4TH QUARTER OF BASE YEAR
GC-PR-2728-001

CONTRACT NUMBER: **N00014-95-D-0048**

REPORTING PERIOD: **September 1, 1995 - November 30, 1995**

REPORT DATE: **December 22, 1995**

RESEARCH ON NAVY-RELATED COMBAT CASUALTY CARE ISSUES, NAVY OPERATIONAL-RELATED INJURIES AND ILLNESSES AND APPROACHES TO ENHANCED NAVY/MARINE CORPS PERSONNEL COMBAT PERFORMANCE

I. INTRODUCTION

This report summarizes the results of GEO-CENTERS' technical activities for the fourth quarter of the contractual base year for the Naval Medical Research Institute (NMRI) under Contract N00014-95-D-0048, Delivery Order #001. This delivery order encompasses a variety of scientific studies that are capable of supporting ongoing and projected programs under the cognizance of NMRI; NMRI TOX/DET-Dayton, OH; NDRI-Great Lakes, IL; the NDRI Detachment-Bethesda, MD; and the National Naval Medical Center-Bethesda, MD.

The format for these periodic technical progress reports consists of four sections each listed by the location of the research. The sections are (1) Descriptions of work to be performed, (2) Objectives planned for the current reporting period, (3) Summary of work performed during current reporting period, and (4) Objectives for the next reporting period. Accumulated scientific reports, technical reports and journal articles are being provided as part of this annual technical progress report. Specifically, the research conducted by GEO-CENTERS during this quarterly reporting period has been focused on the following general scientific programs:

A. Infectious disease threat assessment and enterics programs.
B. Immune cell biology, wound repair and artificial blood studies.
C. Biomedical diving programs.
D. Personnel performance enhancement programs.
E. Breast Care Center.
F. Dental related diseases.
G. Toxicological studies.

GEO-CENTERS, INC.

II. NMRI, Bethesda, MD

A. INFECTIOUS DISEASE THREAT ASSESSMENT AND ENTERICS PROGRAMS

DESCRIPTION OF WORK TO BE PERFORMED

Jendrek

- Scott Jendrek conducts fermentations in a BL-3 suite and depending upon the organism being fermented purification of the product may also be performed. Creates all documentation associated with any aspect of his position, including Standard Operating Procedures, batch records, and any documentation required for newly installed equipment. Scott Jendrek also either installs all new equipment related to his projects or oversees their installation by the technicians sent by the supplier. Assists in the molecular biology aspects of his position, DNA purification, plasmid isolation, electroporation, and other techniques are performed on a regular basis.

Weeks

- Serve as an associate to the principal investigator for a research program involving pathogenic, molecular, and biochemical analysis of bacteria and their virulence factors. Experimentation requires a knowledge and proficiency of laboratory techniques and procedures for performing biochemical and immunological analyses. Conduct surveys of the scientific literature to develop background data on techniques and formulate approaches for the investigations, develop experimental protocols, define the objectives and priorities of subsidiary problems and arrange the details of cooperative investigations with other organizations when necessary. Is responsible for the general administration of the laboratory reagents, solutions, enzymes, and other materials and equipment used to conduct the studies described. Is responsible for the cleanliness and orderliness of working areas, freezers, and refrigerators. Is responsible for the training and orientation of all new laboratory technicians. Organizes and accumulates repositories of bacterial strains, plasmids, enzymes and sera with sufficient documentation of the histories of each. Maintains sufficient stocks of all reagents, supplies, and equipment required for a well organized molecular biology laboratory. Performs other duties as assigned. Immunizations are required.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Jendrek

- Continued with molecular biological aspects of the position and also started to do sequencing gels and work with the actual mutation aspect of the Protective Antigen gene. The shuttle vector will be employed to moving the mutant PA gene into various bacterial species.

Weeks

- The objective for this quarter is to finish the mapping of the pFra plasmid of *Yersinia pestis*. The other objective for this quarter is to finish the characterization of the pFra Mud mutagen library that was started earlier this year.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Jendrek

- Conducted various molecular biological tasks to create a plasmid shuttle vector for the PA gene between *E. coli* and *B. subtilus*. Experiments ranged from plasmid isolation and digestion to ligating fragments of PCR DNA into plasmids. It seems now that the shuttle vector maybe be fatal to the *E. coli* so transformation of the PCR plasmid of PMK3-1 pPA102 KNM2/PA2NC directly into *B. subtilus* is being tried.

Weeks

- Although the mapping of the above mentioned plasmid has been slow to materialize, the actual mapping was started at the end of September and is almost completed. The characterization of the pFra Mud mutagen library is also near completion after several interruptions and impediments.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Jendrek

- Next Quarter it should be known if the plasmid made by PCR can be transformed directly into *B. subtilus* and if so will continue along that line of reasearch. Conditions will be worked out by which factor Xa will cut the mutant protein strains created in the lab. Fermentation will also be performed on these mutant protein producers and they will be purified as well.

Weeks

- The objectives for next quarter are to finish the mapping of the pFra plasmid and to finish the characterization of the pFra Mud library.

II. NMRI, Bethesda, MD

B. IMMUNE CELL BIOLOGY, WOUND REPAIR RESEARCH AND ARTIFICIAL BLOOD PROGRAM

DESCRIPTION OF WORK TO BE PERFORMED

Li

- Lipopolysaccharide is considered to be the responsible agent for the induction of endotoxic shock, affecting the liver and intestine as the target organs. Four intestinal cell lines have been selected for study. For these experiment, cells have been stimulated with various concentration of LPS (endotoxin) and incubated in low oxygen (hypoxia) concentrations to mimic hemorrhagic shock (HS) *in vitro*.

Fan

- Currently conducting molecular biological research at Septic Shock Research Program, Naval Medical Research Institute. Projects include "Detection of inducible nitric oxide mRNA in cardiac myocytes and cardiovascular smooth muscle cells of septic rats" and Regulation of protein kinase C mRNA isotypes expression in rat cardiovascular smooth muscle cells".

Chavez

- At the Blood Research Detachment of Walter Reed Army Institute of Research (WRAIR), the mission is to study aspects of blood research. Major foci in this area include basic research on the physical properties of hemoglobin, red cell storage and preservation, blood banking, and multiple levels of clinical trials on hemoglobin-based blood substitutes. Dr. Chavez's role at WRAIR involves basic research on the properties of hemoglobin and hemoglobin-based blood substitutes and technical assistance for pilot plant operations. Hemoglobin oxidation, toxicity, and nitric oxide binding are some of the current problems in the field of hemoglobin-based blood substitutes. Several projects have been designed to better understand the mechanisms involved in hemoglobin function. This knowledge will hopefully allow for alleviation or elimination of problems associated with hemoglobin-based blood substitutes and lead to a viable blood substitute product.

Ring

- The function of this position has been to provide technical support for biological investigators in the Immune Cell Biology Program.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Li

- An endotoxic and hypoxic intestinal cell model which mimics HS *in vitro* has been established to investigate the cellular cytokine secretion and gene expression in those cells.
- To investigate the mechanisms that oral interleukin-6 (IL-6) improves gut barrier function partially restoring immune function after HS.

Fan

- Continue the investigation on gene regulation of inducible nitric oxide synthase (iNOS) and protein kinase C (PKC) isoforms in rat cardiac myocytes and vascular smooth muscle cells during LPS-induced sepsis using RT-PCR technique.
- Quantitatively analyze changes of iNOS and PKC mRNA levels in above experimental condition using the "mimic" competitive PCR technique.

Chavez

- Completion of ongoing experiments was given the highest priority this quarter.

Ring

- Biological research, as in other areas, involves computers for many activities: data organization, communication, etc. However, many of the investigators are not trained in these disciplines. GEO-CENTERS' presence within the Tissue Bank gives the biological investigators a source of help in resolving technical problems. The objectives for the quarter are not hard scientific accomplishments, but rather support functions which contribute to the overall operation of the Immune Cell Biology Program.

- The first objective is to provide general computer support. Support for stand-alone computers involves assistance with spreadsheets, databases, and operating systems. Support for network connectivity involves hardware installations, software configuration, and personnel training.

- The second objective is to maintain the availability of I.C.B.P.'s calcium imaging system. The system is built around a MicroVax II main processor with an attached Gould image processor Software from R.Y. Tsien (University of California) was used as the software base. Numerous hardware and software enhancements have been done. The result is a system highly specialized for the measurement of intracellular calcium. Use of the system has fluctuated, with active periods and slack periods. The objective is to manage the system during the active periods and maintain a "corporate memory" during the slack periods.

- The general aim of the work has been and continues to be: how can the tools of computing resources be made available to the biological investigator? In imaging, networks, and programming, the objective is to be responsive to the needs of individual investigators and the department as a whole.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Li

- HTC-8, HTB-40 intestinal cells have changed their morphology under low oxygen ($1\%O_2$) incubation with LPS. Using a neutral red assay, a variety of cellular deteriorations have been found in these intestinal cell lines (HTC-8, HTB-40, CaCo2 and IEC-6) induced by LPS hypoxia incubation at different time points. HS mouse intestine (ileum) mucosa epithelia have demonstrated IL-6 and IL-6 receptor positive staining which was stronger than that of normal mouse controls.

Fan

- Generated semiquantitative results of the activation of gene expression of iNOS and certain PKC isotopes in LPS-treated rat cardiac myocytes and aortic smooth muscle cells.
- Obtained preliminary data of quantitative analysis of iNOS mRNA expression.

Chavez

- Heme exchange experiments have been run on native hemoglobin (HbA_0) and *aa*Hb, a chemically modified cross linked hemoglobin being studied as a possible blood substitute. We have extended the studies to include partially oxidized hemoglobins, a condition which occurs *in vivo* and in substantial amounts in animal studies. For comparative purposes, 100% oxidized hemoglobin was used. As with previous results, the *aa*Hb showed less heme loss than HbA_0, a desired property of a blood substitute. Interestingly, with less oxidation present, the rate of heme loss was reduced. This result illustrated that maintaining the iron in the reduced state stabilizes the globin structure. Under anaeorobic conditions, hemoglobin exists in the deoxy form and heme loss is reduced even further. Hemoglobin in the deoxy state stabilizes the tetrameric form. This tetrameric form has been shown to have retarded heme loss. To complete the experiment, heme exchange experiments will be performed with HbA_0 and *aa*Hb a function of concentration. Under these conditions, protein conformational changes can be separated from dimer-tetramer equilibrium. Results will be presented at the 1996 Biophysical Society Meeting.

- Currently, heat denaturation provides a convenient way to both separate unmodified hemoglobin from *aa*Hb as well as sterilize it. The laboratory has investigated the use of heat denaturation to likewise remove unmodified hemoglobin from ββHb, another cross linked derivative. A comparison between *aa*Hb and ββHb *in vivo* will probe the effect of varying oxygen delivery properties. In short, the heat denaturation procedure employed for *aa*Hb will not work for ββHb due to the instability of ββHb under deoxy conditions. These results are being incorporated into a overall paper describing the improvements implemented in the pilot plant within the last year. This paper has been given top priority at the moment, superseding other manuscripts in preparation.

Ring

The mice database program has been organized into three modules:

1. Tracking program for ear tagged mice: tracks status and blood lines
2. Tracking program for caged mice: tracks purchases and experiments
3. Statistics program: calculates aggregate statistics for accounting purposes across all mice databases.

- The simultaneous use of hundreds of mice combined with high "mouse turnover" within experiments resulted in a difficult management situation. The program provides tools to organize experimental usage as well as manage cost control.
- General support was provided in the department's use of NMRI's network.
- General support was provided in assessing and organizing the department's use of commercial software packages.
- Support was provided to run the calcium imaging system for visiting NIH personnel. A paper is being planned.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Li

- Repeat the neutral red assay with 1% O_2 long term incubated intestinal cells using a Laser Doppler technique to investigate alterations of local blood microcirculation in HS rat intestine.

Fan

- Optimize the "mimic" competitive PCR technique.
- Accurately quantitate iNOS mRNA levels in rat cardiac myocytes and smooth muscle cells.
- Continue the investigation of gene regulation of PKC subspecies during sepsis.

Chavez

- The manuscript on liposome encapsulated hemoglobin, in collaboration with the Naval Research Laboratory and Smith Kline Beecham, was rejected by *Science*. The reason given at the time was "not topical." This paper is being resubmitted, most likely to the *Journal of Physiology*. The pilot plant paper will be completed and submitted to a chemical engineering journal. The heme exchange experiments will be completed and presented at the 1996 Biophysical Meeting in February. The heme affinity experiments are slated to be completed in December and manuscript preparation will begin thereafter.

Ring

- Develop, improve and upgrade software programs as needed
- Assist in managing the department's use of the network
- Continue to work with the NIH investigators in calcium studies

II. NMRI, Bethesda, MD

C. BIOMEDICAL DIVING RESEARCH

DESCRIPTION OF WORK TO BE PERFORMED

Cortes

- To conduct surgeries for the Alzheimer's project.
- To perform other surgeries such as cannulations and probe implants.
- To collect and analyze data from animal models.
- To assist in experiments using a hyperbaric oxygen chamber.
- To care and maintain laboratory animals in excellent condition for experiments.

Shea

Alzheimer project:

- To perform micro dialysis experiments in the CNS of rats which have previously been listened at the nucleus basalis Mynert (NBM) via the drug NMDA.
- Analyze the neurotransmitters acetylcholine (ACh), norepinephrine (NE), and serotonin (5-HT), in microdialysis perfusate obtained from the above experiments.

Oxygen toxicity project:

- Run trial microdialysis experiments in the newly designed hyperbaric chambers prepared for 100% oxygen environment under deep dive conditions.

Genetic seizure project:

- A collaborative project within diving medicine where a genetic strain of mice prone to spontaneous seizures will be analyzed for baseline neurotransmitter amounts in various brain regions and compared to normal litter mates.

Free radical project:

- A HPLC method will be set up in order to measure free radical formation in various brain regions of awake animals. Microdialysis samples will contain a free radical trapping agent which will then be assayed via HPLC electrochemical detection.

Obowa

- Provide technical assistance in the Diving Medicine research laboratory investigating exposure to hyperbaric oxygen (HBO) and its effects on the CNS.
- Prepare brain tissues for staining, section tissues using the cryostat, perform immunohistochemical staining methods on tissue sections, care for animals, perform surgical procedures on rats, and order laboratory equipment and supplies.

Porter

- To support in the selection of a hyperbaric CO_2 analyzer for fleet submarine dry deck shelter use.
- To support in the selection of an ambient air CO_2 analyzer for fleet submarine use.
- To support analysis of fleet soda lime for possible contamination and to analyze the samples for specific dye concentrations.
- To assist with other laboratory duties as needed.

Ruby

- Providing gas analysis support for Navy diving studies at NMRI.
- Development of new gas analysis methods in support of Navy Fleet requirements.
- Specification, procurement and installation of laboratory chemical analytical instrumentation to support NMRI/DBTFA gas analysis capabilities.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Cortes

- To finish the Alzheimer's project by including more data points to the curve.
- To continue experiments involving the oxygen chamber.
- To continue the lesion study on the effect of NMDA in the frontal cortex.

Shea

Alzheimer project:

- To continue the experiments in the NMDA listened rats by increasing the number of observations at various time points post lesion.

Oxygen toxicity project:

- A number of rats implanted with microdialysis probes will be run in the new hyperbaric chamber under various depths in order to test the microdialysis equipment under 100% oxygen levels.

Genetic seizure project:

- Eight mice with specific genetic alterations will be sacrificed and their brains dissected into various regions.
- After extraction a number of neurotransmitters will be measured and comparisons made between seizure and non seizure prone animals.

Free radical project:

- A HPLC proceed will be set up to measure and separate the free radical formation of 2,3 dihydroxybenzoic acid and 3,5 dihydroxybenzoic acid, products of free radicals and salicylic acid. The salicylic acid is the trapping agent used in the microdialysis perfusion medium.

Obowa

- Perform immunohistochemical staining of tissue sections for detection of c-fos.
- Set up immunohistochemical assay for detection of heat shock proteins (HSP)
- Refine technique and isolate brain region involved in performing olfactory bulbectomies in rats.
- Continue studies with NMDA lesions of the area tempesta to determine its significance in relation to HBO exposure and seizure activity.

Porter

- To continue with fleet soda lime analysis as samples come in from the manufacture.
- To continue the testing program for the candidate CO_2 analyzers.

Ruby

- Identify, purchase and develop procedures for the use of portable particle size analyzers for measuring the particulates in divers breathing air sources.
- Assist the Naval Sea Systems Command (NAVSEA) in the development of a portable carbon dioxide analyzer capable of operation in hyperbaric atmospheres for use in Dry Deck Shelters (DDS) and host ships.

- Provide technical support to the clean van, the MRCC and the gas farm operations.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Cortes

- Finished and presented the Alzheimer's project at the Neuroscience meeting.
- Assisted on the collection and analysis of data from experiments involving calcium agonists and antagonists drugs.
- Collected and perfused animal's brains for immunocytochemistry.
- Coordinated and supervised the use of the stereotaxic apparatus as well as surgical procedures and protocols.
- Performed trials to test the hyperbaric chamber.
- Tried out a halothane system for future surgical procedures.
- Performed cannulations, probe implants as well as other surgical procedures.
- Cared and maintained animals in optimal conditions.

Shea

Alzheimer project:

- The number of animals at various time points after NMDA lesioning has been increased to four or five per time designated.
- The analysis of neurotransmitters is on schedule and has been included in the results on this animal model of Alzheimers' presented at the Society for Neuroscience in November.

Oxygen toxicity project:

- Equipment for the diving chamber microdialysis experiments has been completed. Testing of this system is to begin soon.

Genetic seizure project:

- HPLC procedures for various neurotransmitters have been established. Normal mice brains have been dissected and tested in these chromatography methods.

Free radical project:

- HPLC conditions have been worked on for the separation of salicylic acid-free radical products.

Obowa

- Performed animal surgeries, including olfactory bulbectomies on rats to refine technique and determine optimal procedure.
- Cut brain sections for HSP and c-fos studies using cryostat. Stained tissue sections for c-fos and HSP detection using immunocytochemistry.
- Performed western blot gel electrophoresis on brain tissue samples for determination of heat shock protein.
- Assisted investigators with dive chamber operation while diving rats for different projects.
- Ordered laboratory equipment and supplies. No further work was done this quarter involving NMDA lesions of the area tempesta.

Porter

- Hyperbaric testing of two prototype CO_2 analyzers for dry deck shelter use is completed. Necessary modifications have been made by the manufacture to produce a commercial unit for fleet use.
- A test program is currently being formulated for the commercial units prior to final recommendation for fleet hyperbaric use.
- 15 buckets of military grade soda lime were tested and approved for fleet use.
- Preformed other laboratory as requested.

Ruby

- Several candidate portable particle size analyzers were evaluated. The MIE Co. MINIRAM unit was selected as the best candidate for field use and the GRIMM Model 1.105 Portable Dust Monitor was selected as a primary standard for calibrating the field units.
- The pre-prototype units for the NAVSEA project, that incorporate the design changes required for hyperbaric use, have completed the initial bank of qualification tests and have received NAVSEA approval for use on submarines. The units have been tested at sea during Seal Team operations. The units performed perfectly.

- Support has been provided, as required, to other functional areas of the diving medicine department.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Cortes

- To revise the Alzheimer's project for publication.
- To incorporate the use of the Hyperbaric chamber and its applications in future studies.
- To collect sufficient data points in the NMDA lesion study.
- To fully use the halothane system as the primary anesthetic in our surgeries.
- To keep and maintain the animals and surgical facilities in excellent condition.

Shea

Alzheimer project:
- Finish the time course study for post lesioning NMDA animal studies.
- Set up halothane anesthesia apparatus for rats in a stereotaxic frame, whereby they can be listened and dialyzed immediately after injection of drug.

Oxygen toxicity project:
- Set up microdialysis procedures in the hyperbaric chambers, especially under high oxygen conditions.

Genetic seizure project:
- Determine the neurotransmitter contents of various brain parts in genetically altered mice and compare these with control litter mates.

Free radical project:
- Set up the HPLC methods to measure the products of free radicals that are trapped by salicylic acid during microdialysis experiments.

Obowa

- Interact with other investigators to refine technique for performing olfactory bulbectomies.

- Perform olfactory bulbectomies in rats to observe any changes in HBO seizure activity and c-fos distribution in the brain following bulbectomies.
- Perform surgeries implanting EEG electrodes after bulbectomies. Continue immunohistochemical staining procedures for both c-fos and HSP identification.
- Compile and evaluate EEG data from HBO exposures for c-fos study and determine if more exposures are needed for this study.

Porter

- To continue analysis of fleet soda lime for contaminates and dye concentration as needed.
- To continue testing program for candidate CO_2 analyzers for both the dry deck shelter and sub ambient air programs.

Ruby

- Assist the Naval Sea Systems Command (NAVSEA) in a study to characterize the volatile organic compounds that may be present in the divers air banks of 688 class submarines when the banks are charged from the sub air after prolonged time at depth without ventilation.
- Continue the development of the NAVSEA candidate carbon dioxide analyzers and the divers air bank sampling procedures.
- Continue the screening and selection process to obtain portable analyzers for field testing of compressors used to produce divers breathing air. Parameters will be oil mist/particulate, oxygen and carbon monoxide.
- Support the needs of the diving medicine functional areas as required.

II. NMRI, BETHESDA, MD

D. PERSONNEL PERFORMANCE ENHANCEMENT STUDIES

DESCRIPTION OF WORK TO BE PERFORMED

Wolf

- Provide management support to the Combat Casualty Care Program at Naval Medical Research and Development Command. Duties include reviewing medical research plans and progress reports, recommending laboratory guidance, evaluating research proposals, drafting periodic and *ad hoc* management reports and developing presentation materials.

McCowin

- Provide management support to the Special Operations Forces Medical Technology Development Program at the Naval Medical Research and Development Command. Duties include reviewing and evaluating medical research proposals, reviewing progress reports and comparing them with the approved research plans, recommending guidance, and drafting periodic and ad hoc management reports and developing draft presentation materials. The scope of research includes all topics within the Special Operations Forces Medical Technology Development Project. This includes investigations relevant to the treatment of disease, trauma, effects of environmental extremes and treatment for medical support of Special Operations Forces operations. In addition, from time to time, collect, process and report findings on critical issues which are directly related to other urgent military medical research Issues within the purview of the Special Operations Forces Medical Technology Development Program.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Wolf

- Continue to make the transition for the new Research Area Manager (Code 45) as smooth as possible.

McCowin

- Collect monthly obligation and expenditure reports from principal investigators
- Collect 3rd incremental progress reports
- Review 3rd incremental progress reports for proposal relevance
- Collect pre-proposals and proposals for new FY96 projects
- Evaluate proposals of new FY96 projects
- Submit FY96 obligations and expenditure report to Special Operation Defense Acquisitions Center (SORDAC) for Execution Review Conference.
- Submit FY95 unobligated funds report to SORDAC
- Assist in slide presentations for SORDAC
- Make arrangements to attend Biomedical Initiative Steering Committee (BISC) meeting
- Prepare 3rd incremental and proposal package and submit to BISC
- Evaluate work unit file of principal investigators for funding and deliverable status
- Evaluate FY97 Task Statements

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Wolf

- Made the transition for the new Director of Research & Development (co-hatted as the Research Area Manager for Combat Casualty Care) as smooth as possible.

- Completed a basic introduction to the new computer hardware and software; including e-mail, scheduling, spread-sheeting, data base and word processing. Attempting to make the transition as easy as possible for my co-workers.

- Reviewed uniformed services university of the health sciences (UHUHS) request for extensions to complete six work units. Then review was completed and recommendations were made to the Director of Research & Development. A response letter to USUHS was prepared and submitted.

- Reviewed the PCWUIS "1498" submissions from in house labs for format and correctness.

- Created a standard funding spread sheet format for all research area managers based on Director of Research & Development concept of data elements.

McCowin

- Collected monthly obligation and expenditure reports from principal investigators
- Collected and reviewed 2nd incremental progress reports for proposal relevance
- Updated Work Unit Information Summary (WUIS) in the Defense Technical Information Center (DTIC) database
- Collected pre-proposal, proposals and WUIS for new FY96 investigators and reviewed documents for Special Operations Forces (SOF) relevance
- Evaluated proposals of continuing FY96 projects
- Prepared Research & Development Testing & Engineering (RDT&E) budget submission for FY96 and FY97
- Submitted FY96 initial obligations report to Special Operations Defense Acquisitions Center (SORDAC)
- Submitted FY95 unobligated funds report to SORDAC
- Created U.S. Special Operations Command (USSOCOM) guidance package, which contained dates, guidelines, formats and instructions for preparing and submitting the required documents.
- Prepared slide presentation format for U.S. Special Operations Biomedical Research and Development Program Review

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Wolf

- Attempt to identify those areas where GEO-CENTERS, INC. may assist NMRDC in the transition.
- Enhance workers capabilities with the new local area network at NMRDC.

McCowin

- Attend BISC in December 1995
- Collect and evaluate 3rd incremental progress reports
- Collect monthly obligation and expenditure reports from principal investigators
- Submit monthly obligation and expenditure reports from SORDAC

- Solicit and evaluate pre-proposals for FY97
- Plan to attend March 95 BISC

E. BREAST CARE CENTER, Bethesda, MD

DESCRIPTION OF WORK TO BE PERFORMED:

Patient Service Representatives
Hamilton, Jenkins, Williams

- Develop a system for processing and interviewing patients. Establish uniform policy for check-in/check-out procedures.
- Devise a system for completion and collection of third party patient insurance forms.
- Use standard patient registration procedures.
- Perfect receiving patients and incoming telephone calls/inquiries, determine priorities and refer to proper person/department.
- Develop a schedule to ensure all mammogram films would be pulled 1-2 days ahead of current clinic schedule.
- Ensure that all incomplete patient records and third party forms are corrected or returned to proper staff for completion/correction.
- Set up records and filing system for paperwork associated with each patient record. Ensure that all documents processed are in accordance with department standards and that all forms are in designated order in the patient records. Label files for storage as permanent shadow files.
- Assist with establishment of standard operating procedures.
- Orient new support team members and clinical team staff to office routine.
- Devise a system of notifying all no-shows, record information in shadow file and initial.
- Call/notify all physicians the day before they are scheduled for clinic; let them know approximately how many patients they will see.
- Ensure that all mail is picked up/delivered daily.
- Print the Composite Health Care System (CHCS) daily schedule and end of day reports.

Balintona

- Responsible for addressing the psychosocial status, mental status, patient concerns, and the impact of diagnosis on family relationships of breast cancer patients.
- Facilitation of the Stage I & Stage II Breast Cancer Survivors' Group

- Organization and planning of the Advanced Breast Cancer Group and Partners' Group which will begin this coming quarter. The planning includes soliciting patient participation, development of a brochure to distribute to patients and staff education about the availability of the group.
- Collect and analyze research data on the adjustment and social support in male spouses based on the support group.
- Liaison with the National Naval Medical Center Social Work Staff. Coordinate individual and group psychotherapy based on Social Work assessment and clinical intervention needed.

Fields

- Performed technical services in the Mammography Department including mammograms, assisting in biopsies, quality control and record keeping.

Higgins

- Responsible for all patient flow activities.
- Acts as Relief Clinical Nurse Manager, in the absence of the Nurse Case Manager.
- Responsible for opening and closing all clinical areas and preparing exam rooms for patient use.
- Check crash cart in APU on designated day.
- Triage telephone calls and walk-ins.
- Reviewing and sorting mammogram reports, ordering new labs/radiographs in CHCS as per doctor's orders.
- Gathering all results for patient visits.
- Schedule all nursing lunch assignments.
- Processing linen and hazardous material within the Center.
- Check and order supplies for clinical exam rooms and needle/syringe cart.
- Coordinate all FNAs and procedures and notify Nurse Case Manager of all such cases.

Lopez

- Develop and integrate a breast care educational program for female Department of Defense beneficiaries and their support persons.
- Develop an education program to include all breast care issues with an emphasis on early detection of breast cancer.

- Provide pre-operative teaching and educate patients regarding breast cancer and treatment options.
- Be available as an information resource person for the patient and their support person.
- Assist in coordination of staff development programs under the direction of the nurse case manager.
- Acting as relief Ambulatory Care Nurse under the direction of the nurse case manager.

McIntyre

- Support a newly designated research program which focuses on breast cancer.
- Perform nursing and managerial duties.
- Liaison between the Radiology Department Mammography Section, the Breast Care Center (BCC), and other hospital departments.

Mitchell

- Assist in the development and opening of the Breast Care Center at the National Naval Medical Center.
- Provide care for patients with both malignant and benign diseases of the breast, including: initial evaluation, definitive medical or surgical therapy, and long term follow-up.
- Develop a data collection system which will allow patient data to be collected in a manner which will permit clinical research to be performed at a later date.
- Educate medical students, residents, nurses, and other physicians in the diagnosis and treatment of malignant and benign diseases of the breast.

Wallace

- Coordinate administrative activities of the Breast Care Center (BCC).
- Manage patient/physician schedule templates in the Composite Health Care System (CHCS).
- Collect and report monthly workload statistics.
- Collect and report monthly man hours reports.
- Become proficient in use/manipulation of CHCS

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Hamilton, Jenkins, Williams

- Streamline and organize front-desk procedures.
- Retrieve and ensure completion of third party insurance forms.
- Improve routing and response to incoming telephone calls/inquires.
- Use standard registration procedures for all forms pertinent to each patient.
- Provide roster for pulling mammogram films 1-2 days prior to the scheduled visit.
- Coordinate policies for scheduling appointments/procedures for patients calling/walk-ins/consults/cards.
- Streamline physician schedule notification process.
- Refine CHCS daily schedule and end of day reporting.
- Ensure pick-up and delivery of mail in a timely manner.

Balintona

- Development of a research proposal.
- Decided on the measurement tools and sample size
- Liaison with Dr. Miller, Nursing Researcher at USUHS, for feedback on the research proposal.
- Addressed the psychosocial status and individual concerns of patients in the Breast Care Center.
- Co-facilitated the Breast Cancer Survivors' Group.

Fields

- To attend the 10th annual update in mammography conferences, as a second Lorad stereo static biopsy in service.
- Acquire more experience with all the procedures in the department.
- Strive to strengthen her working relationship with co-workers.
- Coordinate mammography formations between the BCC and the Radiology Department.

Higgins

- Preparing clinical areas for patient care.
- Develop inventory checklist for ordering clinical supplies and needle/syringes.

- Investigate and establish par levels for clinical supplies.
- Stock all clinical areas with par level supplies.
- Identify nursing roles for the Ambulatory Care Nurse.
- Further develop computer skills utilizing Windows '95 software, especially CHCS.
- Evaluating videos and teaching materials for breast self exam, with the Clinic Educator.
- Development of nursing documentation and care plans tools.
- Evaluate patient teaching material in Spanish and provide interpretation for Clinical Educator.
- Organization of triage area and triage files.
- Continue to further develop breast self exam teaching in a timely and effective manner.
- Utilizing past radiology reports to improve clinical impression reporting for mammograms/cxrs/ultrasounds.
- Creating a patient package for cancer follow-up patients, to include cxr, mammogram and blood work prior to visit.

Lopez

- To assist in set up of work environment.
- Assist in identifying the role of Ambulatory Care Nurse and Clinical Nurse Educator.
- Assess educational needs and access information resources.
- Obtain basic instructional materials.
- Initiate breast self exam teaching program.
- Develop documentation tool for patient teaching.

McIntyre

- Assist the Radiologists/staff with stereotactic and ultrasound guided breast biopsy procedures.
- Perform assessments on all stereotactic biopsy patients and provide these patients with post breast biopsy teaching instructions.
- Assist in developing the critical pathways in nursing.

Mitchell

- Open the Breast Care Center and provide comprehensive care to patients with diseases of the breast.

Wallace

- Develop and maintain schedule templates for Breast Care Center attending physicians in five sub-clinics.
- Develop BCC clinic profiles in CHCS for five sub-clinics.
- Coordinate with Radiation Oncology and Medical Oncology Clinics to aid in the establishment of a working relationship with the BCC.
- Train four Patient Service Representatives.
- Develop a structure for collection of third party insurance procedure and diagnosis codes.
- Collect and Report monthly man hours and workload.
- Obtain training in CHCS.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Hamilton, Jenkins, Williams

- Began organization of front-desk procedures.
- Processed and interviewed patients through CHCS and designated forms, obtained and updated all patient demographic information and ensured completion of forms.
- Obtained and verified pertinent insurance information utilizing available forms. Obtained third party insurance forms from physicians at end of each visit.
- Required identification card from each patient and imprinted all clinic forms pertinent to that patient.
- Received patients and incoming telephone calls/inquiries, determined priorities and referred to the proper source.
- Explained clinic procedures to patients.
- Retrieved/returned Mammogram films daily.
- Ensured completion of incomplete patient records and third party insurance forms.

- Set up records and maintain filing system for paperwork associated with each patient record. Ensured that all documents processed are in accordance with department standards. Filed all forms in designated order in patient record.
- Labeled files for permanent shadow files.
- Scheduled and coordinated front desk procedures in accordance with department policy.
- Oriented new support team members and clinical team staff to office routine.
- Participated in team planning to assure team members meet team quality standards. Maintain department standards of productivity.
- Notified physicians the day before they are scheduled for clinic; let them know approximately how many patients they will have.
- Picked-up and delivered mail daily.

Balintona

- Completed the abstract for the Partners' of Breast Cancer Support Group Study.
- Decided the appropriate sample size and measures for the independent and dependent variables.
- Developed the conceptual framework.
- Designed the outline of each Partners' Group Session.
- Addressed the psychosocial status, mental status and patient/family concerns in the Breast Care Center.
- Co-facilitation of the Stage I & Stage II Breast Cancer Survivors' Group Development of the Social Work Assessment tool and critical pathways.
- Worked closely with the BCC Nurse Case Manager to provide seamless care to patients.
- Liaison with the National Naval Medical Center Social Work Department.

Fields

- Attended the mammography conference and also the Lorad stereo static in service, acquiring 23 continuing education points, needed for her state radiology license.
- Continued to gain more experience with all the procedures of the department especially the stereo static biopsies.
- Made a concerted effort to improve her working relationship with all co-workers. The BCC is open and a new working relationship has been established between the BCC and the mammography department.

Higgins

- Coordinated patient flow activities in the clinical areas with patients, nurses and doctors.
- Created inventory checklist and established all par levels for clinical supplies.
- Stocked all exam rooms and clinical areas, appropriately.
- Further identified the nursing assignments as an Ambulatory Care Nurse.
- Coordinated all FNAs and procedures and notified the Nurse Case Manager of such procedures.
- Triaged telephone calls and walk-ins. Also organized triage area and files.
- Decreased time of breast exam teaching, while remaining effective.
- Improved written clinical impression on radiology order request.
- Processed linen and hazardous material.
- Developed results folder and cancer follow-up patient package.
- Worked with Clinical Educator to select videos for patient viewing and teaching, in Spanish and English.
- Orientation of new nurses and doctors to patient flow processes and documentation within the Breast Care Center.
- Participated in multi-disciplined meetings, to further enhance the relationship between BCC and Radiology.

Lopez

- Prepared examination rooms in anticipation of clinic opening.
- Assisted as team member in the identification of duties for triage nurse, procedure nurse, team leader, and nurse educator.
- Functioned as ambulatory care nurse providing breast self exam teaching to all patients seen in the clinic.
- Assisted the physician with physical exams, procedures, collection and scheduling of diagnostic tests.
- Obtained teaching materials to meet patient needs.
- Accessed Internet to obtain educational and informational materials.
- Submitted initial and secondary draft of teaching documentation tools.

McIntyre

- Supervised other mammography personnel.
- Coordinated mammography scheduling.

- Maintained six month mammogram follow-up on a monthly basis.
- Obtained mammography statistical data for FDA purposes.
- Correlated mammography and pathology findings via the CHCS.

Mitchell

- The Breast Care Center opened on October 11, 1995, with over 100 patient visits in the month of October. The number of patient visits continues to increase, and should exceed 200 in the month of November.
- Provide care to 148 patients.
- Performed 20 biopsies and 15 major surgical procedures.
- Medical students and residents have been integrated into the Breast Care Clinic and are actively involved in the care of patients with diseases of the breast.
- A system to collect patient data has been developed, and data is being recorded in a manner which will allow for retrospective clinical research to be conducted at a later date.

Wallace

- Developed schedule templates for BCC attending physicians in all sub-clinics.
- Developed BCC clinic profiles for all sub-clinics.
- Established training schedule for all Patient Service Representatives. Three of four have completed training successfully, one Patient Service Representative is currently in training.
- Developed a successful working relationship with the Radiation Oncology Clinic.
- Developed a form for collection of third party insurance procedure and diagnosis codes.
- Took training courses for CHCS, currently practicing to gain proficiency.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Hamilton, Jenkins, Williams

- Streamline and organize front-desk procedures.
- Coordinate scheduling with other clinics for smoother follow-up visit for the patient.
- Maintain department standards.

- Attend classes involving CHCS training as well as computer training.
- Develop User Defined Keys for greater efficiency in completing tasks which require routine keystrokes.
- Assist in development of a personal development program to assure continuing professional growth.
- Assist in development of standard operating procedures.

Balintona

- Begin the Partners' Support Group in November. Administer the appropriate pre and post-measurement scales to the partners (men) for research purposes.
- Continue with the development of the Advanced (Metastatic) Breast Cancer Group.
- Liaison with Dr. Ken Miller, Nursing Researcher, for further feedback and input on the research proposal.
- Submission of the Research Proposal to the Clinical Investigation Department (CID).
- Provide social work services to patients by addressing psychosocial status, mental status, patient and family concerns.
- Coordinate individual, family and group psychotherapy for patients.
- Work closely with the other Breast Care Center to coordinate patient care.

Fields

- Continue to fine tune skills in the various procedures and modalities in the department. The new interface with the BCC will be expanded and strengthened.
- Continue using patient understanding and a team effort approach with her co-workers to make for a happier healthier work environment.
- Continue to expand her knowledge of mammography and breast diseases.

Higgins

- Refine breast self exam teaching and monitor patient's teaching needs.
- Continue to enhance nursing knowledge base on breast cancer issues.
- Continue to further develop personal computer skills.
- Attend a seminar/conference on breast cancer issues.
- Continue to work with Clinical Educator to screen patient teaching materials in Spanish and English.
- Function as team member to develop nursing care plans and documentation tools.

- Improve patient flow processes in the clinical area.
- Completely stock all exam rooms with par level supplies.
- Further develop inventory procedure with the nursing team.
- Continue to orient new staff members to patient flow processes and forms within the Center.

Lopez

- Continue to setup educational resource room.
- Continue to identify educational materials needed and obtain approved materials.
- Participate in round table discussions to streamline patient flow while meeting patient needs in a timely and effective manner.
- Participate in NNMC health fair providing information on Breast Care Center, breast cancer prevention and early detection.
- Screen video tapes and begin setting up resource library for patient use.
- Provide pre-operative, breast cancer, treatment options, and breast self exam teaching to patients and their support persons.
- Initiate teaching documentation forms when approved.

McIntyre

- Continue to perform nursing and managerial duties, as described above.
- Become more proficient in utilizing the Breast Care Center Task Manager computer system.

Mitchell

- Continue to provide care for patients with both benign and malignant diseases of the breast at the Breast Care Center. Increase the number of patients seen in the Breast Care Center to approximately 400 patient visits each month.
- Expand the educational opportunities available at the Breast Care Center by developing a curriculum for medical students and physicians to receive intensive training in the management of breast diseases over a 2 to 4 week period at the Breast Care Center.
- Develop protocols for both retrospective and prospective clinical research involving patients with breast cancer.

Wallace

- Continue to build a relationship with the Radiation Oncology Clinic.
- Establish a successful working relationship with the Medical Oncology Clinic.
- Manage schedule templates for all sub-clinics of the BCC.
- Develop Standard Operating Procedures Manuals for the BCC.
- Become proficient in use of CHCS.

III. NDRI, Great Lakes, IL and NDRI Detachment, Bethesda, MD

A. DENTAL DISEASES-RELATED RESEARCH

DESCRIPTION OF WORK TO BE PERFORMED

Turner

- To continue to procure blood samples from Naval recruits, and to isolate polymorphonuclear neutrophils (PMNs) from the blood samples.
- To extract the granule contents from the PMNs.
- To begin experiments focused on the bactericidal activities of the PMN granule extracts.

Lamberts

- To assist as an editorial consultant in the preparation or review of manuscripts to be submitted for publication.
- To aid in the preparation of research presentations (such as posters) for scientific meetings, and in the review of research proposals, research communications (letters, rebuttals), etc.

Ahlf

- To act as liaison between the scientists at the Naval Dental Research Institute (NDRI) and varied research sites.
- To determine effectiveness of existing dental preventive program, while working as Principle Investigator of project entitled "Development of Multimedia Programs for Dental Prevention and Treatment."

Miller

- Provide technical assistant with ongoing Immunology research projects.
- Maintain and upgrade the laboratory such that the research experiments are carried out smoothly.

- Maintain and record proper technical procedures and data produced for each experiment.

Beck

- Responsible for all aspects of Immunological and Microbiological activities within the Naval Dental School. This includes the development and supervision of research protocols, dental resident mentoring activities, instruction of courses in dental microbiology and dental immunology. Serve as a link between NIH sponsored research and Naval Dental Research programs, and troubleshooting of research programs, computers, instrumentation and equipment.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Turner

- To adapt methods for growing facultative anaerobic bacteria, using modified media in an aerobic environment.
- To achieve "transfer blot" technology by use of an enhanced chemiluminescent system.

Lamberts

- To continue work on the preparation of a manuscript on a new bacterial medium.
- To assist Naval Dental Research Institute (NDRI) investigators editorially whenever requested.

Ahlf

- To coordinate logistics and authorization for permission to collect clinical samples from research sites.
- To evaluate the effectiveness of dental preventive education at Great Lakes Naval Base.
- To investigate expanded function courses and strategies utilized by the Navy.
- To assist with logistics for Clinical Investigation Projects.

Miller

- Become very familiar with two dimensional gel electrophoresis system. Run two dimensional gels on gingival crevicular fluids from various donors. Learn to analyze the patterns of two dimensional gels using "BioImage two dimensional analysis" computer software.
- Continue gathering samples for RNA analysis of stimulated cells using Polymerized Chain Reaction (PCR).
- Proceed with immunofluorescence stains of lymphocytes for flow cytometry analysis.

Beck

- Work Unit: 0601152N.MR00001.001-0063. Evaluation of the influence of superantigens and polyclonal B-cell activators in periodontal disease. To continue work on this IR project and bring it to closure by September, 1995. The major focus will be on the flow cytometry and rt-PCR procedures and on stimulator characterization. We have in operation a new two-dimensional gel electrophoresis system and hope to utilize it for characterization of superantigens.
- Work Unit: 0601152N.MR00001.001-0063. Influence of *Treponema denticola* on cytokine production by cells obtained from periodontally diseased and non-diseased individuals. Finalize data for presentation at the 3rd International Cytokine Conference.
- Work Unit: 0601152N.MR00001.001-0063. Long term frozen storage of lymphocytes. Final manuscripts need to be completed.
- Work Unit 63706N.M0095.006-3014. Influence of growth factors on gingival and periodontal ligament fibroblasts. A main goal will be to complete writing of the manuscript.
- To initiate a new program entitled "Evaluation of disproportionate expression of T-cell receptor V_ regions in lymphocytes from patients with advanced periodontitis". We anticipate beginning these studies as soon as all final approvals are obtained from SRC and from the Human Use Committee.
- To develop a 2-D electrophoresis procedure to study components of gingival crevicular fluid from individuals with severe periodontitis. In addition, time will be spent on training in Bio Image Software in order to be able to analyze 2-D protein patterns.
- Work Unit: 0601152N.MR00001.001-0063. IL-6 production by polymorphonuclear leukocytes resident in periradicular and periodontal lesions. Collection of tissue samples and preliminary identification of IL-6 will be made.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Turner

- Continued adapting the column chromatography (SMART) system to requirements (buffers, sample sizes, etc.) of our studies.
- Refined the electrophoresis system further to enable better analyses of micro samples generated in our experiments.

Lamberts

- Reviewed manuscripts for LCDRs J. Simicek and W. Deutsch.
- Reviewed three abstracts (Pederson, Ralls, Schade) of research studies that will be presented at the March, 1996 meeting of the International Association for Dental Research.
- Reviewed galleys of a manuscript on "Salivary Levels of Alpha-2 Macroglobulin, Alpha-1 Antitrypsin, C-Reactive Protein, Cathepsin-G and Elastase in Human Subjects with or without Destructive Periodontal Disease" that is now in press for publication in the Archives of Oral Biology.
- Continued work on the preparation of a manuscript for E. Pederson et al. on a new bacterial medium. Work on this manuscript has been constrained by the need for additional experimental data.

Ahlf

- Collected plaque and saliva samples for evaluation.
- Received approval as Principle Investigator of Prevention Program project.
- Began exploring educational methodologies that are being utilized to determine effectiveness.
- Observed and evaluated the wellness lecture for female recruits.
- Assisted in coordinating logistics for two of Clinical Investigation's projects.
- Facilitating consultant's contracts.
- Arranging and contracting completion of repair work on research-related equipment.
- Developing flow charts for the progress of research projects.
- Provided in-service training for the Recruit Training Command's staff on oral cancer.
- Develop plan of action to implement and evaluate the effectiveness of an expanded functions program at Great Lakes.

Miller

- Tremendous progress has been made separating proteins using the techniques of two dimensional gel electrophoresis. Proteins of gingival crevicular fluid, bacteria, and mammalian cultured cells have been separated two dimensionally. Currently, the gingival crevicular fluid samples are being studied to improve and reproduce the patterns. This will set the basis for the future experiments that will deal with comparing patterns of gingival crevicular fluid from diseased and non diseased subjects.
- Aside from two dimensional patterns, these samples were also run on mini gels for whole band analysis.
- Lymphocytes subjected to the antigens such as Td 33521, Td 33520, and anti CD3 were isolated for both flow cytometry and PCR studies. Some cells were stained with monoclonal antibodies specific for Vß receptors on cells. Receptors of interest included Vß2, Vß3, Vß8, and Vß13.1. Cells stimulated with specific Vß regions were identified and quantified using flow cytometer. Each cell group was compared with one another. Remainder of cells in each group were frozen away for the identification of Vß positive cells using PCR method.
- In addition, blastogenesis experiments were performed to measure cellular activation upon stimulation.
- Several cell lines are being maintained for continuous growth such that cells from these lines can be used for further experiments.

Beck

- Relative to the project Evaluation of the Influence of Superantigens and Polyclonal B-cell Activators in Periodontal Disease (Work Unit 0601152N.MR00001.001-0063) we have utilized quantitative reverse transcriptase PCR procedures to identify superantigen production in bacterial preparations by identifying specific V__message production by lymphocytes exposed to bacterial superantigens. This procedure incorporates the use of a state-of-the art fluorescence gene sequencing procedure and involves a collaboration between this laboratory and the National Institutes of Health Epidemiology and Disease Prevention Branch of NIDR (Dr. Scott Diehl). Our work has continued the show that extracts of oral bacteria such as Actinobacillus actinomycetemcomitans, Prevotella intermedia, and Treponema denticola are able to stimulate expansion of T-cells carrying TCR-V_1 and V_8 mRNA. A manuscript reporting these results is in the final stages of preparation.

- Work Unit: 0601152N.MR00001.001-0063. IL-6 production by polymorphonuclear leukocytes resident in periradicular and periodontal lesions. Tissue samples have now been collected from 12 donors and thin sections have been evaluated for IL-6 production utilizing an enzyme immunoassay procedure.
- Work Unit 0601152N.MR00001.001-0063. No additional work has been done concerning the project involved with the influence of *Treponema denticola* on cytokine production by cells obtained from periodontally diseased and non-diseased individuals, other than the evaluation of data. Results from this study have been presented at the 3rd International Cytokine Conference in Harrogate, UK, in September.
- Work Unit: 0601152N.MR00001.001-0063. Relative to freeze storage of lymphocytes all bench work has been completed and the first manuscript has been written and submitted for publication.
- Relative to the project "Influence of growth factors on gingival and periodontal ligament fibroblasts." we have completed all of the planned research and a rough draft of a manuscript has been completed.
- Relative to the program entitled "Evaluation of disproportionate expression of T-cell receptor V_ regions in lymphocytes from patients with advanced periodontitis", eight of the expected twelve control subjects have been identified and peripheral blood lymphocytes obtained and stored in anticipation of mRNA extraction.
- Relative to the develop a 2-D electrophoresis procedure to study components of gingival crevicular fluid from individuals with severe periodontitis, preliminary studies have been completed insuring that the basic procedure is operable. In addition, training in the use of Bio Image Software for analysis of 2-D protein patterns has also been completed.
- A manuscript entitled "Antimicrobial activity of dentin bonding systems and glass ionomers" relating work done on the evaluation of glass and ceramic materials has been submitted and accepted for publication in *Journal of Operative Dentistry*.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Turner

- Begin alternate procedures for determining bactericidal activities of PMN granule extracts.
- Procure purified PMN factors from commercial sources.

- Make further refinements in isolation, fractionation and purification of PMN granule extracts.

Ahlf

- To continue exploring educational methodologies relative to dental preventive measures.
- To follow plan of action and milestones regarding the dental prevention program.
- To assist with tobacco cessation and related research projects.
- To determine direction to take at Great Lakes with the expanded functions program.
- To initiate the expanded functions program and determine its effectiveness.

Lamberts

- Complete, if possible, work on the medium manuscript. This goal depends, in large part, on the acceptability of data still being acquired for this research report.
- Assist NDRI investigators editorially whenever requested.

Miller

- Two dimensional gel electrophoresis will be the primary focus of upcoming quarter. More samples will be gathered to evaluate and compare the patterns of gingival crevicular fluid.
- Additional experiments for flow cytometry and PCR studies will be done.

Beck

- Work Unit: 0601152N.MR00001.001-0063. Evaluation of the influence of superantigens and polyclonal B-cell activators in periodontal disease. To complete work on one additional manuscripts. This will bring the project to completion.
- To continue work on a new program entitled "Evaluation of disproportionate expression of T-cell receptor V_ regions in lymphocytes from patients with advanced periodontitis".
- Work Unit: 0601152N.MR00001.001-0063. Long term frozen storage of lymphocytes. Complete writing of the second of two final manuscripts.
- Work Unit 63706N.M0095.006-3014. Influence of growth factors on gingival and periodontal ligament fibroblasts. Complete preparations of manuscripts.

- To begin to expand the procedures used in 2-D electrophoresis to more fully study the various proteins found in gingival crevicular fluid.
- To continue development of *in-situ* rt-PCR hybridization procedures for identification of IL-1 and IL-6 in clinical tissue samples.

IV. NMRI TOX/DET, Dayton, OH

A. TOXICOLOGICAL STUDIES

DESCRIPTION OF WORK TO BE PERFORMED

Briggs

- Department Manager for the NMRI Contract at the Toxicology Detachment.
- Performs toxicology studies as an Associate Investigator and as the Senior Contractor Representative.
- Quality Assurance Officer to assure compliance with research standards and AAALAC certification requirements.
- Collaborates the GEO-CENTERS resources to perform toxicology research as tasked by the Officer-In-Charge for meeting the mission objectives.

Bowen, Kimmel, Reboulet

- Conduct aerosol characterization and inhalation toxicity of Spectrex Fire Extinguishant (SFE).
- Develop engineering plan for mixed combustion gases inhalation toxicity studies.
- Develop analytical methodology for mixed combustion gases inhalation studies.
- Develop analytical and generation methodology for ozone depleting substances replacement (ODSR) and ozone depleting substance (ODS) neurobehavioral studies.

Smith, Zepp

- Conclude the range finding and multiple dose study for Spectronix Fire Extinguisher (SFE) Formulation A.
- Conduct the an edema study using SFE Formulation A.
- Develop, refine and validate biochemical assays for the micro plate. These assays include acid phosphatase, alkaline phosphatase and total protein.
- Complete characterization studies on second generation of SFE formulation A.
- Initiate studies on developing a new screening procedure for the determination of cardiac sensitization.

IV. NMRI TOX/DET, Dayton, OH

A. TOXICOLOGICAL STUDIES

DESCRIPTION OF WORK TO BE PERFORMED

Briggs

- Department Manager for the NMRI Contract at the Toxicology Detachment.
- Performs toxicology studies as an Associate Investigator and as the Senior Contractor Representative.
- Quality Assurance Officer to assure compliance with research standards and AAALAC certification requirements.
- Collaborates the GEO-CENTERS resources to perform toxicology research as tasked by the Officer-In-Charge for meeting the mission objectives.

Bowen, Kimmel, Reboulet

- Conduct aerosol characterization and inhalation toxicity of Spectrex Fire Extinguishant (SFE).
- Develop engineering plan for mixed combustion gases inhalation toxicity studies.
- Develop analytical methodology for mixed combustion gases inhalation studies.
- Develop analytical and generation methodology for ozone depleting substances replacement (ODSR) and ozone depleting substance (ODS) neurobehavioral studies.

Smith, Zepp

- Conclude the range finding and multiple dose study for Spectronix Fire Extinguisher (SFE) Formulation A.
- Conduct the an edema study using SFE Formulation A.
- Develop, refine and validate biochemical assays for the micro plate. These assays include acid phosphatase, alkaline phosphatase and total protein.
- Complete characterization studies on second generation of SFE formulation A.
- Initiate studies on developing a new screening procedure for the determination of cardiac sensitization.

Prues

- Finding replacements for the current ozone depleting fire extinguishants has prompted the Navy to perform studies on alternative products, such as, the Spectrex Fire Extinguishant (SFE).
- Because of the toxic effects of chemicals on one's ability to perform their duty, the Navy is involved in trying to ascertain the mode of action of certain toxicants (i.e. TMPP), the presence with which their personnel are likely to come into contact

Narayanan, T.K., Jung

- Trimethylolpropane (TMPP) Evaluation
- Identification of the brain receptor for TMPP
- Measurement of the clearance rate of TMPP from the body
- Work on the Cell Model Project.
- Culture liver cells (WB 344) to be used for the cell model project
- Prepare a standard curve for a fluorescence assay for cellular glutathione content
- Perform neurotransmitter analysis
- Set up an HPLC for the analysis of the neurotransmitter samples
- Preparation of a standard curve

Ritchie

- Serve as Assistant Group Leader for the Neurobehavioral Toxicology Group at the Tri-Service Toxicology Consortium and NMRI/TD and as Associate Principal Investigator (API) for all currently funded neurobehavioral toxicology-related work units.
- Assist in all areas of program management, budgetary control and procurement, research design, protocol preparation, research supervision, statistical analysis and preparation of scientific papers and abstracts in the area of neurobehavioral toxicology research.

During the current quarter, Dr. Ritchie has continued research in six areas:

1. Non-Lethal Measures of Toxicity: Performance Decrements (WU.1409).
2. Development and validation of a comprehensive neurobehavioral toxicity assessment battery (The Navy NTAB) . . . Replaced (10/95) by Neurobehavioral Toxicity Assessment Battery (NTAB): Assessing Animal Responses to Pharmacological Challenge (WU.1605): Predictive validation of the NTAB by comparison of animal

and (known) human responses to identical pharmacological challenges on neurobehavioral tests with topographical similarity.

3. Improved Methods for Evaluating Performance Deficits Induced by Brief Exposures to High Concentrations of Gases or Vapors (WU .1408): Neurobehavioral effects of exposure to low concentrations of Ozone-Depleting Substances (ODSs) and Non-Ozone Depleting Substance Replacements (ODSRs) of military interest.
4. Improved Methods to Evaluate Performance Deficits Induced by Complex Mixtures (WU.1420): Neurobehavioral effects of exposure to low concentrations of single and mixed combustion gases as might be encountered in military scenarios.
5. TMPP Mechanisms of Action: Development of Neurobehavioral molecularization techniques (WU.1512): Development of a number of neuro-molecular (cellular-level) analytical techniques for eventual inclusion in the Navy Neuro-Molecular Toxicity Assessment System (The NTAS); anatomical disposition and effects of trimethylolpropane phosphate (TMPP), a potent neurotoxicant produced through the pyrolysis of synthetic lubricants used in military ships and aircraft.
6. Persian Gulf War (PGW) simulation using Sprague-Dawley rats (U.S. Army and NMRI/TD): Development of an animal model to simulate exposures encountered by Persian Gulf War veterans.

Martin

- Editing all technical reports, manuscripts, proposals, standard operating procedures and other materials for presentation from NMRI/TD's staff and Tri-Service Toxicology.
- Ensure these materials are cleared through the appropriate military channels.
- Serving as a liaison to procurement personnel in acquiring manuscripts reprints.
- Ensuring these publications and presentations are documented properly for reference purposes.
- Providing editorial assistance as a representative of NMRI/TD in the Tri-Service Toxicology Program Development Committee.
- Ensuring that authors are provided with the appropriate guidelines and instructions for formatting their publications.
- Developing and expanding the technical communication program at NMRI/TD and Tri-Service Toxicology under the direction of the Officer-in-Charge of NMRI/TD.

Binole, Rix

- Upgrade servers to NTS 3.51 and upgrade Library server to Pentium server and NTS 3.51 Operating System.
- Implement Domain Names Server (DNS) and network wide anti-virus program.
- Install System Management Server (SMS) and SQL on server.
- Continue maintenance of servers and backup of data files.
- Continue to provide hardware/software technical support for TOXDET personnel.
- Develop support software where needed.

Ademujohn

- The purpose of the neuro behavioral laboratory coordinator at NMRI/TD is to provide technical support to various aspects of ongoing on-site projects in neurobehavioral research. During this quarter the coordinator has been and will be involved in testing the effects of Ozone-Depleting Substances (ODS) refrigerants, specifically, Halon 1301 on animal models via computer-aided qualitative and quantitative methods. The coordinator also supervises animal training protocols for the modified Wahmann chamber inhalation studies and roto-wheel studies.

Connolly

- Cataloging print and non-print materials for circulation.
- Ordering and maintaining serials collection.
- Handling reference questions.
- Providing interlibrary loan assistance.
- Locating needed materials in other libraries.
- Preparing book orders.

Caldwell

- Director of Project Development for Air Force and Army toxicology research.
- Project Director for the Persian Gulf Veterans Research Project.
- Principal Investigator and member of the Inhalation Toxicity of Vapor Phase Lubrication project team.
- Principal Investigator and member of the Combustion Toxicology project teams dealing with Advanced Composite Materials used on aircraft (e.g., B-2 and F-22).

Walsh

- Member of the Pharmacodynamics Technical Area Group.
- Perform as Project Director for the Inhalation Toxicity of Vapor Phase Lubrication project.
- Support *in-vitro* toxicology research.
- Perform as Project Director for the Combustion Toxicology, Munitions and Propellants project.

Geiss

- Group Administrator for the PharmacoDynamics (PD) Technical Area Group, Tri-Service Toxicology Consortium.
- Ensure that PD support is provided to project leaders.
- Continue methods and protocol development for the molecular biology laboratory.
- Perform ongoing experiments for the 60-day Trichloroethylene (TCE) study and Environmental Initiative (EI).

McDougal

- Lead basic and applied research in dermal penetration and biologically-based mathematical modeling.
- Responsible for all aspects of quantitative dermal toxicology and dermal risk assessment for Army, Navy and Air Force at Tri-Service Toxicology Center.
- Primary investigator on an Air Force Office of Scientific Research project entitled, "Species Differences in Skin Penetration".
- Lead multi disciplinary research group (University of Michigan, North Carolina State University, Colorado School of Mines, and University of California at San Francisco) addressing quantitative dermal toxicology for Air Force Chemicals.

Grabau

- Provide image analysis support to the multi-disciplinary toxicological research activities at Tri-Service Toxicology.

Narayanan, L.

- Evaluate hydrochloric and sulfuric acid in field samples using HPLC coupled with conductivity detector in support of the "Health Effects Of Gel-Cell Batteries Study."
- Measure Phospholipase-C and phosphocholine cytidylyltransferase (CTP), activity in control and perfluorocarboxylic acids exposed rats.
- Detect and quantitate Ammonium Perchlorate using HPLC coupled with conductivity detector.
- Measure thyroglobulin (hTg), reverse triiodothyronine (rT3), and iiodothyronine(T3), and Thyroid stimulating hormone (TSH) levels in control and Ammonium Perchlorate exposed rats.

TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

Briggs

- Facilitate the resources to keep current projects and programs on schedule, maintain and improve productivity when and where it is needed, and identify opportunities for growth including the preparation and evaluation of new pre-proposals.
- Continue to perform on the Executive Steering Committee of NMRI/TD to manage the contractor technical and support services resources and respond to tasking by the Officer-In-Charge (OIC).
- Perform project reviews to assure the quality and integrity of the data that is collected and reported. Specific activities and accomplishments are presented in the next section.

Bowen, Kimmel, Reboulet

- Complete multiple dose SFE inhalation toxicity study using guinea pigs.
- Determine analytical and generation requirements for Halon 1301™ inhalation exposures.
- Develop engineering plan for the safe storage and utilization of mixed combustion gases.
- Prepare Society of Toxicology abstracts for poster presentations.
- Prepare Wahmann inhalation exposure chamber and door (roto-rod chamber).
- Prepared and submitted pre-proposal for Adult Respiratory Distress Syndrome (ARDS) project.

Smith, Zepp

SFE Formulation A:

- The objective of this research is to evaluate the potential health effects of exposure to the by-products of pyrolyzed SFE. SFE is fire suppressant and a potential replacement for Halon 1301.

Cardiac Sensitization:

- The objective of this research is to develop an *in vitro* test for the determination of cardiac sensitization. These initial studies will set the basic background needed for future studies.

Prues

Technical support for the following NMRI/TD Projects is to be provided:

- Spectrex Fire Extinguishant (SFE)
- Trimethylolpropane Phosphate (TMPP)
- Serve as contract representative on the Safety Policy Committee

Narayanan, T.K., Jung

- TMPP continue the characterization of the TMPP receptor in the brain measure the rate of clearance of TMPP from the body
- Preparation of the final DBNP technical report
- Maintain the cell line (WB 344) to be used for the cell model experiment
- Prepare a standard curve of oxidized and reduced glutathione for a fluorescence assay
- Set up the Beckman HPLC for the chromatographic analysis of the neurotransmitter studies

Ritchie

Non-Lethal Measures of Toxicity: Performance Decrements (WU.1409):
Work unit funding terminated 31 August 1995.

- To complete development and initial evaluation of a number of neurobehavioral tests for inclusion in the Navy NTAB.
- To complete experimentation outlined in WU.1409.
- To begin data analysis and writing of several journal articles for publication.

- To write and submit a major animal use protocol to support FY 96 experimentation mandated in new WU.1605.
- To assist in the orientation of newly-assigned Neuropharmacologist LT Alan Nordholm.

Improved Methods for Evaluating Performance Deficits Induced by Brief Exposures to High Concentrations of Gases or Vapors (WU.1408):

- To initiate spectral discrimination testing of White Carneaux pigeons during exposure to CFC-12 and HFC-134a.
- To identify a source of Halon-1301 and HFC-227ea for subsequent toxicity testing of rats and pigeons.
- To begin operant training of 40 new rats for subsequent testing.

Improved Methods to Evaluate Performance Deficits Induced by Complex Mixtures (WU.1420):

- To complete initial engineering of a complex system for exposure of rats & pigeons to up to five fire gases simultaneously.
- To complete procurement of a Perkin-Elmer FTIR System 2000 and appropriate gas cells.
- To identify an appropriate external consultant for system development.
- To complete and submit a WPAFB ACUC animal protocol for initial experimentation.

TMPP Mechanisms of Action: Development of Neurobehavioral Molecularization Techniques (WU.1512):

- To assist Dr. Lindsey with development of surgical techniques for implantation of cannulas and electrodes in rats for EEG/microdialysis recording during direct micro infusion of TMPP into the nucleus accumbens.
- To assist Dr. Lindsey in development of a simultaneous microdialysis/EEG/ behavioral seizure analysis system.
- To assist with surgical implantation of multi-unit stimulating and recording electrodes in the nucleus accumbens and ventral tegmental area in twenty rats.
- To assist Dr. Narayanan with investigation, using competitive radio labeled binding techniques, of the specific CNS receptor binding of TMPP.
- To complete study of the effects of low dose exposures to TMPP on performance of a well-learned operant habit in rats.
- To assist in completion of three scientific posters for presentation at the 1995 Society for Neuroscience meeting in San Diego, CA.

- To acquire DEA Schedule II and IV drug licenses.
- To prepare abstracts for the 1996 SOT meeting and 1996 NEHC conference.

Persian Gulf War (PGW) simulation using Sprague-Dawley rats (U.S. Army and NMRI/TD):

- To rewrite and re-submit WPAFB ACUC protocol detailing scientific research to be conducted FY96-98.
- To procure and pilot study apparatus to be used for neurobehavioral evaluation of exposed rats.

Martin

- Continue to develop technical communication program at NMRI/TD.
- Continue to draft strategic plan for Tri-Service Program Development committee.
- Prepare marketing materials and briefing packages for NMRI/TD.
- Learn new desktop publishing and multimedia software recently purchased by NMRI/TD.

Binole, Rix

- Continue to add and improve our network capabilities.
- Continue to evangelize our internet capabilities to the toxicology community.
- Complete conversion of Library server to NTAS 3.5.
- Install System Management Server (SMS).
- Install Domain Name Server (DNS).
- Convert current Navy Supply database to SQL or FoxPro.
- Continue to provide technical support for TOXDET personnel.
- Develop support software where needed.

Ademujohn

- Testing various ODS substances (Halon 1301) on animal models using diminished capacity as the endpoint in Carneaux pigeons.
- Range finding using operant - trained animals and measuring subsequent stages of diminished capacity.
- To compile, catalog and computerize the above mentioned data.
- To train pigeons for problem solving protocols
- Daily maintenance of pigeon intake and logging performance results.

- To obtain operant testing and training data for animals used in operant exposure testing
- To organize, catalog and generate computer graphics, cumulatively from the above mentioned data.
- To maintain data for future reference in upcoming publications.
- To be responsible for the procurement of all materials used in testing and training protocols.
- Responsible for documenting and maintaining operant weights.
- Responsible for writing standard operating procedures for pigeon training protocols.
- Responsible for making daily accurate and detailed entries and updates of all work unit laboratory books.
- Responsible for compiling information for and conducting weekly meeting with / between work unit P. I.'s and laboratory technicians.

Connolly

- Catalog materials as received.
- Catalog materials not yet cataloged.
- Prepare renewal list for serials.
- Provide library service to the toxicology community at WPAFB.
- Set up program of records for library.
- Start working on a manual card catalog.

Caldwell

- Prepare a manuscript and oral presentation of ammonium perchlorate work for JANNAF meeting.
- Prepare an abstract for the 1996 Society of Toxicology meeting.
- Prepare a Project Director folder for the Persian Gulf Veterans Research Project.
- Present results from Vapor Phase Lubrication project at national meeting.
- Submit results from Vapor Phase Lubrication to peer reviewed journal.
- Prepare technical report of combustion studies of ACM used on the B-2 aircraft.
- Prepare Animal Use Protocol for Persian Gulf Veterans research project.

Walsh

- Prepare a manuscript and overhead presentation for JANNAF.
- Prepare a Project Director folder for the Vapor Phase Lubrication Project.

- Perform a literature search for Phase II, Vapor Phase Lubrication Project.
- Prepare an extended summary for Tribologist Annual Meeting.
- Provide technical support to the Regulation of Metallothionein Gene Expression project.
- Prepare a Project Director folder for the Combustion Toxicology/ F22 project.

Geiss

- Identify needs in molecular biology research support and design a technical approach to fulfill those needs.
- Develop protocols and research methods for the evaluation of biological effects of Air Force-related materials.
- Cooperate in current research relating to the toxicological effects of trichloroethylene (TCE), its metabolites and other compounds.
- Develop Molecular methods for the Environmental Initiative (EI).
- Train other scientists in molecular biology research methods.

McDougal

- Submit abstract for SOT on *in vitro* diffusion cell.
- Start pharmacokinetic studies with hairless guinea pigs for the AFOSR grant.
- Start to use animal and/or human skin in diffusion cells.
- Assess the penetration of the Modular Artillery Charge System in diffusion cells.

Grabau

Species Differences in Skin Penetration:
- Develop methods to study early events of dermal inflammation utilizing advanced imaging technologies and three-dimensional modeling.

Combustion Toxicity of Advanced Materials (ACM):
- Investigate and develop methods to conduct analysis and modeling of *in-vitro* and *in-vivo* combustion studies.

Collaborative Hydrazine Study:
- Develop and conduct new image analysis algorithms to quantify immuno-histochemical markers in tissues used for evaluation of the toxic effects of hydrazine

Program Development:

- Assist in the development of innovative strategies to promote research advancement and development at Tri-Service Toxicology as a member of the Tri-Service Product Development team.

Image Analysis Program Development:

- Initiate a program for the utilization of new image analysis hardware and software to include; training of primary and secondary users, integration of assets into active research efforts, and develop research collaboration opportunities outside of Tri-Service Toxicology
- Support any projects requesting image processing and analysis support.

Narayanan, L.

Ammonium Perchlorate:

- Standardize the elution conditions using anion exchange column for perchlorate detection and quantitation of ammonium perchlorate using HPLC coupled with conductivity detector.
- Estimate thyroglobulin (hTg), reverse triiodothyronine (rT3),and triiodothyronine (T3) levels in control and ammonium perchlorate exposed rats, using radioimmunoassay (RIA).
- Measure thyroid stimulating hormone (TSH) levels in control and ammonium perchlorate exposed rats, using RIA.

Basic Field Research:

- Extract and quantitate chloride and sulfate anions in field samples provided in solid sorbent tubes using HPLC, coupled with conductivity detector.

Hepatotoxicity Studies:

- Measure phosphocholine cytidylyltransferase (CTP), and phospholipase-C activity in control, perfluorodecanoic acid (PFDA), and perfluorooctanoic acid (PFOA) exposed rat livers.

SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

Briggs

HFC 236fa refrigerant replacement project:

- Reviewed the developmental toxicology report in rats and reported the negative findings to the Senior Scientist.
- Reviewed the 90-day inhalation toxicology study in rats and reported the negative findings to the Senior Scientist.
- Co-authored two abstracts that were submitted to the Society of Toxicology.

Cardiac sensitization project:

- Assisted in the preparation and coordination of the four protocols for conducting cardiac sensitization projects and assured funding support.
- Initiated study start ups and assured that resources were available to meet the objectives of the contracts.

DBNP project:

- Prepared protocols for conducting the developmental and reproductive toxicology studies.
- Wrote the statement of work for the Request For Proposal.
- Reviewed the response to the statement of work and assisted in the selection of the successful bidder for those studies.

Defense Women's Health Research Program:

- Collaborated with NMRI/TD in assuring that resources are available to conduct two research projects in response to the extramural announcement.
- Prepared a facility expansion paper to provide the requirements for a new inhalation toxicology laboratory. Traveled to Research Triangle Park, NC to evaluate the inhalation resources at the EPA laboratory and the Chemical Institute of Industrial Toxicology (CIIT). This information was provided to the Air Force and is being considered for expanding our current inhalation resources.
- Assisted with the AAALAC certification inspection that occurred on November 13. The inspection was conducted to assure compliance with the "Guide for the Care and Use of Laboratory Animals". This inspection is conducted once every three years and is essential to the mission of NMRI/TD. The veterinarians from AAALAC indicated

that no corrections were needed, and we can expect full Accreditation when the Council meets in January.

- Assisted with the preparation of the Annual Animal Care and Use Report to Congress and assured compliance with the regulations.
- Prepared four pre-proposals for future research at NMRI/TD.
- Assisted with the preparation of two additional pre-proposals.
- Reviewed eight additional pre-proposals and assisted with the improvements of these research proposals and ranked them for the OIC.
- Reviewed Standard Operating Procedures and reviewed current methods for assuring compliance with operating standards.
- Prepared and submitted a technical article for publication.

Toxicology:

- Assisted with project management and functioned as a member of the Executive Steering Committee (ESC)at NMRI/TD.
- Conducted validation procedures for methods used for the SFE project.
- Attended the Lessons on Leadership conference.
- Made preparations to attend the Society for Risk Analysis meeting.
- Attended the annual training for handling radioactive materials.
- Attended the annual animal handlers training program.

Bowen, Kimmel, Reboulet

- Developed mathematical model to calculate theoretical growth curves for SFE aerosol particles as a function of relative humidity.
- Completed experiments to determine particle growth, particle size and distribution as a function of relative humidity and nominal concentration.
- Drafted and submitted three abstracts for 1996 SOT annual meeting to be held in Anaheim, CA. The submitted abstracts were acknowledged and accepted (refer to attachments).
- The inhalation exposure chamber housing the roto-rod apparatus was fitted for a new door and leak checked in preparation for ODS/ODSR performance decrement neurobehavioral exposures to Halon 1301™ and hydrofluorocarbon (HFC) 125. Multiple dose inhalation toxicity exposures of SFE to guinea pigs were completed. Nominal exposure concentrations were 35g of test material combusted per cubic meter of exposure volume.
- The ARDS pre-proposal was updated to meet current proposal format requirements and submitted.

Smith, Zepp

- Organization of data books for SFE pilot studies I and II, and range-finding /multiple-dose study.
- Validation of the micro plate assays derived from the macro scale kits purchased from Sigma and Pierce. These enzyme assays included: acid phosphatase, alkaline phosphatase, b-glucuronidase and lactate dehydrogenase from Sigma, and total protein from Pierce.
- SFE Range finding study -- exposure of rats and guinea pigs to increasing concentrations to determine maximum limits.
- Perform enzyme assays, cell viability, total cell count and differential cell count on bronchioalveolar lavage (BAL) from exposed animals.
- Quantified the particulate size and concentration during each exposure.
- SFE Edema evaluation study -- exposure of rats to the highest nominal concentration the manufacturer will use to extinguish fires and euthanize selected animals over a 7-day period to determine the formation of edema.
- Perform enzyme assays, cell viability, total cell count and differential cell count on bronchioalveolar lavage (BAL) from exposed animals.
- Several cardiac sensitization studies were imitated to study the mechanical and elecrophysiological events leading to ventricular fibrillation.

Prues

SFE Project - Range Finding Study Brachoalveolar Lavage:

- Cell viability's performed on the cellular component of the lavage sample.
- Prepared slides for differential counting.
- Compiled and presented research data.
- Developed a surgical procedure for sample collection from test animals being used in to the upcoming blood analysis portion of the study.

TMPP Project - Simultaneous microdialysis perfusion and EEG recording with behavioral observations to determine its effects.

- Performed surgical procedures for the precise implantation of microdialysis guide cannulae and placement of EEG electrodes.
- Setup and collect data during microdialysis perfusion/EEG experiments.
- Assist in data analysis.
- Maintain and stock laboratory supplies.

Narayanan, T.K., Jung

TMPP:

- The receptor that TMPP binds to in the brain was tested by using competitive binding assays. The $GABA_A$, $GABA_B$, and benzodiazepine receptors were tested for TMPP affinity. It was found that TMPP binds to the benzodiazepine receptor. The clearance rate of TMPP was also measured by injecting a rat i.p. with 2 mCi of ^{14}C-TMPP. Blood was taken at selected time intervals from an incision in the tail. Urine and feces were collected as well. By analyzing the counts contained in these samples, it was determined that TMPP is quickly excreted from the body. Nearly 75% of the counts injected had been excreted within the first 24 hours.

Cell model:

- An assay to measure the amount of reduced and oxidized glutathione was standardized. It is a fluorescence assay with an excitation maximum of 345 nm and an emission peak of 425 nm.

Neurotransmitter Study:

- Samples for neurotransmitter analysis were prepared by homogenizing the brain in 0.17 M $HClO_4$ (100 mg tissue/1 ml). This solution was centrifuged and the resulting supernatant was used for analysis. An HPLC is being set up and standardized to run the neurotransmitter samples.

Ritchie

Experiments on Neurological Activity:

- A paper "The NMRI/TD Behavioral Seizure Identification Scale" is awaiting final editing and internal approval for submittal to *Epilepsia*. [1408, 1409, 1420].

Measures of Motor System Integrity and Endurance:

- The journal article "The NMRI/TD Roto-Wheel: A new apparatus for multiple measures of physical incapacitation" [1408, 1409, 1420] could not be completed due to inadequate data from a malfunctioning servo-feedback motor. The article will be completed and submitted to *Behavior Research Methods, Instruments & Computers* as soon as apparatus repairs are completed.
- The journal article "Incapacitation and recovery from brief exposure to ozone-depleting substance replacement HFC-134a [1408, 1409] is awaiting internal clearance for submittal.

Measures of Sensory Acuity, Including Color Vision:

- Eleven white Carneaux pigeons have completed extensive operant training in preparation for ODS/ODSR exposures. Five pigeons have been exposed to CFC-12 or HFC-134a (4-16%) during performance of the spectral discrimination. Repeated emesis in exposed animals has eliminated the pigeon as an animal model for ODS/ODSR evaluation.
- Ten new pigeons arrived on 11 August 1995. Inability of the VS to eliminate parasites and reduce animals to target weight delayed shaping of pigeons until 30 November 1995.

Measures of Social Factors & Emotionality:

- Two juvenile rat play analysis apparatuses have been built; one was customized for use in a Hinners-type chamber during exposure to toxic atmospheres. This apparatus features an 8 x 8 photobeam array for additional analysis of locomotory activity. Incorporation of this apparatus into whole body inhalation exposure chambers has begun.

Measures of Motivational Level & Frustration:

- A progressive ratio operant task has been developed and tested with rats, clearly differentiating motivational deficits associated with toxic exposures from motor system and higher cognitive deficits [.1408, .1409, .1420]. Associated data was presented at the 1995 meeting of the Joint Casualty Group, Dayton, OH, and has been submitted in abstract form to the 1996 SOT meeting and 1996 NHEC meeting.

Measures of Spatial & Temporal Discrimination:

- A Morris Water Maze (MWM) is currently being developed for analysis of toxicant-induced deficits in spatial discrimination [.1408, .1409, .1420].

Measures of Higher Cognitive Decrement:

- Working with a new employee to develop a classical conditioning test (conditioned paw withdrawal) in rats to be included in the NTAB.
- A journal article entitled "Neurobehavioral toxicity of brief exposure to ozone-depleting substance replacement HFC-134a as measured by operant performance" has been written and is awaiting internal clearance for journal submission [.1408, .1409, .1420].

Chemical Kindling Studies [.1516]:

- Contract research at Bowling Green State University (BGSU) during replicated a previously reported result indicating that bi-daily administration of four sub-convulsive doses of TMPP (0.20 mg/kg) sensitizes rats to a subsequent amphetamine challenge, as measured by increase in locomotory activity, 30-60 days following the TMPP administration. TMPP-induced sensitization has also been demonstrated in "sniffing" of hamburger meat experiments.
- A journal article has been accepted for publication in *Toxicology*, 1995. "An overview of chemical and electrical kindling and CNS sensitization phenomenon."
- TMPP Deposition and Clearance [.1516]
- Kennedy-Krieger Institute in Baltimore, MD was contracted to complete sophisticated receptor-select autoradiography studies with TMPP. Initial results indicate that TMPP may allosterically enhance binding of radio labeled AMPA at non-NMDA receptors in the dorsolateral thalamus and areas of the frontal/olfactory cortex in rats. Kennedy-Krieger has, however, failed to complete additional contracted research and is presently being approached by the base contracting legal office.
- TMPP has been shown to bind competitively to the benzodiazepine site of the GABA-A receptor. Final analysis of TMPP binding, differentiating binding effects as benzodiazepine antagonist versus inverse agonist, requires two new radioactivity licenses that are currently awaiting NRC approval.

Microdialysis [.1516]

- Performed surgury for a study involving simultaneous microdialysis, EEG and behavioral seizure investigation of 20 rats, following repeated administration of TMPP, with microdialysis cannulas implanted in the nucleus accumbens. Direct perfusion of TMPP induced hyperlocomotion in addition to a number of stereotyped behaviors, and "kindled" EEG paroxysms in the cortical EEG. Intraperitoneal injection of TMPP "kindled" EEG paroxysms in the nucleus accumbens. Results were presented in a poster at the 1995 Society for Neuroscience meeting, and will be presented at the 1996 NEHC and SOT meetings.

Neurobehavioral Teratology [.1516]

- Protocols were approved at BGSU for the neurobehavioral teratology study outlined in the work unit proposal. Pregnant dams will be injected bi-daily with TMPP throughout the 21-day pregnancy. Rats have been ordered.
- Assisted the approval ACUC approval on a protocol to develop brain tissue slice methodology for evaluation of single cell response to TMPP.

In Vivo Neurophysiology/Neuropharmacology [.1516]

- Assisted in the set-up of a complex multi-unit stimulation and recording system utilized to detect CNS sensitization effects (in nine limbic and sensory pathways) from repeated systemic administration of TMPP.
- Assisted in the surgical preparation of 20 rats with electrodes in the nucleus accumbens and ventral tegmental area.

Other Neurobehavioral Studies [.1516]

- Attention Deficits: A series of studies have been replicated at NMRI/TD demonstrating that administration of sub-convulsive doses of TMPP (0.20 mg/kg, but not 0.10 mg/kg) reliably disrupted a well trained operant habit in rats. This disruption was further shown to be temporally consistent with the appearance of i.p injected TMPP in the brain, and with the observation of paroxysms in the EEG. This study has developed a new method for determining the onset of neurobehavioral effects induced by an injectable drug or toxicant.
- Classical conditioning: Assisted in the preparation of an animal use protocol to investigate effects of repeated TMPP administration on classical conditioning in the rat.
- A WPAFB ACUC protocol detailing planned exposures was initially disapproved, and has been rewritten and resubmitted to comply with ACUC criticisms. Approval is expected in Dec '95.
- A Defense Women's Health Research Program (DWHRP) proposal "Evaluation of combat stress reactions in women using an animal model" was written and submitted.

Martin

- Continued to edit all NMRI/TD publications, presentations, protocols, standard operating procedures, etc.
- Routed publications and presentations through clearance processes.
- Prepared briefing package for NMRI/TD including complete history, staffing, financial and project-related information.
- Prepared draft abstract book for NMRI/TD including abstracts published from 1985 through 1995.
- Outlined and drafted strategic plan for Program Development committee.
- Compiled NMRI/TD 1995 Command Historical Report.
- Assisted in compiling NMRI/TD quarterly report.
- Assisted in preparing information for U.S. Surgeons General Report.

- Assisted in responding to NMRDC request for platform-specific command information.
- Participated in NMRI/TD support service time course study.

Binole, Rix

- All main servers updated to NTS 3.51.
- Library server conversion completed.
- DNS service is now on-line.
- SOPHOS network virus scan software installed.
- Internet home pages added to allow for easier viewing with VGA.
- Database conversion has been delayed pending input by end users.
- SMS has been delayed due to current restrictions on system access.
- Procurement of all software/hardware for use at NMRI/TD.
- Technical support for all software/hardware installed.

Ademujohn

- Compiled , organized, cataloged ,via computer-aided graphics, the weekly data on Pigeon 'Match' protocols .
- Trained and conditioned new and incoming rodent and pigeon groups to protocol adaptation.
- Crucial in the reorganization overhaul of the Neurobehavioral Laboratory to provide a professional appearance and more efficient use of space.
- Implemented an SOP on the procedures for pigeon "Match" protocol training.
- Wrote procedures for Negative Pressure Acquisition in the Wahmann Chamber for pigeon conditioning.
- Maintenance of all laboratory work unit notebooks .
- Implemented several data methods to compile training data and weight maintenance on the pigeon operants.
- Modified pigeon Wahmann chamber for uniform problem solving display.
- Implemented an effective Wahmann chamber disinfection program.

Connolly

- 116 books cataloged and prepared for circulation.
- 148 articles obtained from local libraries.
- 35 articles entered into the reprint database and file.

- 15 interlibrary loans obtained.
- 10 books obtained from local libraries.
- 7 literature searches conducted using in-house CD-ROM database capabilities.
- 6 searches successfully conducted on the internet for customers.
- 2 people trained in search techniques for CD-ROM searching.
- 2 people trained in search techniques for the internet.
- 35 reference questions answered.
- 29 telephone inquiries on journal locations in local area handled successfully.
- 85 bound journal volumes accessioned and stamped.
- 79 sets of cards prepared on existing books for the new manual card catalog.
- Record program set up to track, by title and year, usage of journals, in house, and from other sources.

Caldwell

Persian Gulf Veterans (PGV)Research Project:

- Prepared and had approved an Animal Use Protocol for toxicology research to investigate multiple chemical exposures and psychological stress as potential cause(s) of PGV illnesses.
- Served as thesis advisor for Air Force graduate student performing review of literature on reported health effects of PGV at the Air Force Institute of Technology.

Inhalation Toxicity of Vapor Phase Lubricant project:

- Submitted the final Technical Report.
- Co-authored an extended summary for presentation at the Tribologist Annual Meeting, May 1996.
- Co-authored a manuscript for presentation at the JANNAF Meeting, December 95.
- Presented research results at the Metalworking Fluids Symposium, Dearborn, MI, 15 Nov 95.
- Prepared an article for submission to the *American Industrial Hygiene Assoc. Journal.* Combustion Toxicology/ B-2 project:
- Prepared a draft technical report of combustion study results for project sponsor.

Walsh

Inhalation Toxicity of Vapor Phase Lubricant project:

- Submitted the final Technical Report.

- Prepared and submitted an extended summary for presentation at the Tribologist Annual Meeting. (May 96)
- Prepared and submitted a manuscript and overhead presentation for presentation at the Annual JANNAF Meeting. (December 95)
- Prepared a phase plan which contained essential activities and responsibilities to accomplish this project. In support of this project, performed a literature search essential for the preparation of an animal use protocol.

Regulation of Metallothionein Gene Expression project:
- Performed liver perfusions for primary hepatocyte isolation.
- Performed analytical assays to include; glutathione, microculture tetrazolium, protein and ethoxyresorufin assays.
- Coordinated supply and animal orders.

Combustion Toxicology/ F22 project:
- Prepared a phase plan which contained essential activities and responsibilities to accomplish this project.

Geiss

Laboratory Methods:
- Oligonucleotides were designed, synthesized and used for PCR analysis.

Group Administrator:
- Assist in planning resource allocation, individual training and professional development for the 16 members of the PD group.
- Communicate with other scientists and project leaders to identify their technical needs and to suggest solutions.

TCE and EI:
- Evaluation of potential DNA clones for usage in evaluation of mRNA was performed. Performed plasmid transformation, DNA purification, restriction enzyme digestion and gel electrophoresis of the clones.

General Lab Participation:
- Authored a comprehensive Site-Specific Spill Response Plan (AF-OI-127-9) to ensure compliance of Tri-Service Toxicology with the Base Environmental Management regulations.

- Significant effort over most of this reporting period was put into inventorying, labeling, documenting, and storing materials and waste in the effort to bring the Lab into compliance.
- Organized the PD group to assist in fulfilling this hazardous materials and waste mission.

McDougal

Air Force Office of Scientific Research (AFOSR) project (Dermal Penetration):

- Presented our modeling efforts as part of the Tri-service Toxicology Roundtable on Mathematical Modeling on 6 September 1995.
- Submitted a abstract for the annual Society of Toxicology meeting covering this work.
- Worked with a collaborator from North Carolina University, who visited our laboratory on 25-26 September to learn tissue extraction and analytical techniques for volatile chemicals.
- Attended the Gas Chromatography troubleshooting course in Columbus, Ohio on 27 September.
- New AFOSR proposal received high marks by the reviewers and was approved for funding for the next three years (approximately 160K/yr).
- Presented a talk entitled "Physiologically-Based Pharmacokinetic Modeling of Skin Absorption using Dermal Subcompartments" on 24 October in Charlotte, North Carolina at the Annual Society for Industrial and Applied Mathematics meeting.
- Visited one of our collaborators (Dr Patrick Williams) at North Carolina School of Veterinary Medicine to compare and contrast methods of modeling dermal absorption.
- Visited the staff at Duke University Medical Center on 26 October to discuss the applications of magnetic resonance microscopy for imaging dermal absorption and tissue damage.

Modular Artillery Charge System (MACS) project (Dermal Penetration):

- Assigned as project leader for this project to estimate the absorption of the primary components of this propellant across human skin.
- Completed project management documents for this project which will last for about 8 months.
- Collected cadaver skin from Wright State University (WSU) for histological examination of condition before using in static diffusion cells.
- Investigated methods for dermatoming skin to the appropriate thickness.

- Completed 5 diffusion studies with dibromomethane (as a prototype chemical) to determine flux and permeability constants.
- Wrote three physiologically based mathematical models for the static diffusion cells and in the process of model discrimination and validation.
- Submitted a abstract for the annual Society of Toxicology meeting covering this work.

Other Scientific and Regulatory interactions:

- Presented "Quantitative Methods for Dermal Penetration" as an invited speaker at the American Chemical Society Symposium entitled "Emerging issues in the Risk Assessment Process" in Atlanta on 20 September.
- Presented a talk on our research to the WSU first year Biomedical Science graduate students as part of a course entitled Introduction to Research on 12 October.
- Co-authored a chapter on Dermal Absorption for an International Life Science Institute - Risk Science Institute working group on the "Estimation of Dermal and Inhalational Exposures to Contaminants in Drinking Water".
- Attended WSU School of Medicine Research Committee meetings to rank proposals for seed grants and establish methods for implementing new grant procedures.
- Taught two lectures (opiate analgesics and nonsteroidal anti-inflammatory drugs) to the WSU Nursing students on 31 October.
- Agreed to act as scientific mentor for Capt. Wade Weismann, a newcomer to our laboratories, for the next three years.

Grabau

Species Differences in Skin Penetration:

- Explored new research methods (i.e., magnetic resonance microscopy) and potential collaboration for investigation of dermal absorption kinetics
- Visited Dr. Allan Johnson and staff at Duke University Medical Center on 26 October to discuss the applications of magnetic resonance microscopy for imaging dermal absorption and tissue damage.
- Evaluated methods to measure dermal adnexal components of human skin that are not present in research animals.

90-Day Nose-Only Inhalation Toxicity of Trifluroiodomethane (CF3I) To Male and Female Rats (Study F-37) Thyroid Image Analysis:

- Image processing and analysis methods were developed and allowed quantification of thyroid follicular colloid changes that were dose related and statistically significant.

Collaborative Hydrazine Study:

- Collaborated with Dr. John A. Tinberell (Toxicology Department, School of Pharmacy, University of London, London WC1N1AX, UK) and Dr. John Latendresse to draft "Immuno-histochemical Assessment of Hydrazine Exposed Rats".
- Developed new image processing methods utilized to measure immuno-histochemically detected changes in renal and hepatic tissue.

Toxicity Evaluation of Ammonium Perchlorate Administered in the Drinking Water of Sprague-Dawley Rats:

- Evaluated thyroid changes resulting from perchlorate toxicity using image processing and analysis methods.

AO5 Combustion Program:

- Recommended formulation and incorporation of advanced image processing and analysis studies of *in-vivo* and *ex-vivo* pulmonary tissues exposed to combustion products of advanced composite materials (ACM) into the combustion program.
- Conducted multiple consultations to structure research data from the initial ACM combustion studies to allow for development of simulation models.

Establishment of Isolated Hepatocyte Cultures From Medaka (*Oryzias latipes*) and The Assessment of Trichloroethylene Metabolism by Medaka Liver Preparations *In-Vitro:*

- Coordinated with The Center for *In-Vivo* Microscopy (Duke University Medical Center) to create three-dimensional (3D) compartment models of Medaka.

Preimplantation Effects of Ammonium-Dinitramide Administered in the Drinking Water of Sprague-Dawley Rats:

- Recommended and incorporated formulation of 3D visualization, image processing and analysis of data obtained by confocal microscopy.

Program Development (PD):

- Formulated, completed and presented a strategic plan to integrate the PD team efforts with multiple levels of Tri-Service Toxicology (TST) to the TST management.
- Actively developing Internet assets, informatic assets and preparing displays for up-coming conferences.

Image Analysis Program Development (a.k.a., Scientific Visualization and Analysis):

- Attended meetings with TST personnel to formulate strategies for implementation of advanced scientific visualization and analysis concepts.

- Expanded the concepts of scientific visualization to advanced data visualization and computer-based communications.
- Coordinated the expansion of a new imaging assets user base.

Narayanan, L.

Ammonium Perchlorate:
- Standardized the elution condition for ammonium perchlorate detection using anion exchange column.
- Quantitated ammonium perchlorate using HPLC coupled with conductivity detector.
- Quantitated Triiodothyronine (T3), reverse triiodothyronine (rT3) and thyroglobulin (hTg) levels in Control and Ammonium Perchlorate exposed rats.
- Radiolabelled thyroid stimulating hormone (TSH) antigen with ^{125}I using Chloramine-T method.
- Quantitated TSH levels in control and ammonium perchlorate exposed rats using RIA.

Basic Field Research:
- Extracted and quantitated chloride and sulfate anions in field samples provided in solid sorbent tubes, using HPLC coupled with conductivity detector.

Hepatotoxicity Study:
- Measured Phopholipase C activity and CTP levels in control and perfluorocarboxylic administered rats.

Waste Management:
- Attended and completed "Resource Conservation Recovery Act (RCRA) and Department of Transportation (DOT)" training courses.

GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

Briggs

- Finalize the rabbit developmental toxicology study and assist with the initiation of the exposures.
- Initiate the range-finding developmental and reproductive toxicology studies with DBNP when the funding becomes available.

- Finalize the Standard Operating Procedures and gain approval from NMRI/TD management.
- Prepare and present an abstract for the Naval Environmental Health Center Conference
- Complete the initial phase of the cardiac sensitization project and present the findings at the Society of Toxicology (SOT) meeting.
- Attend the SOT meeting and present the findings from the HFC 236fa and HFC 236ea projects.
- Attend the Society for Risk Analysis meeting.
- Continue to explore the possibility for additional inhalation toxicology space.
- Continue to assist the ESC with improving productivity.
- Continue to prepare the Quality Assurance presentation.

Bowen, Kimmel, Reboulet

- Completion of inhalation toxicity exposures of test animals to SFE for the edema section of the project. Test animals will be exposed to atmospheres of SFE including 60 minute exposures to nominal chamber concentrations of 80b of test material combusted per cubic meter of exposure volume.
- Continued work on the development and initial implementation of an engineering plan for the proposed mixed combustion gases neurobehavioral inhalation toxicity exposures.
- Initiation of the neurobehavioral inhalation toxicity exposures of test animals to the ODS and ODSR gases Halon 1301™ and HFC 125, respectively.

Smith, Zepp

Start the following SFE protocols:

- Protocol 1: Evaluation of Blood Gas, Blood pH, Hemoglobin, Bicarbonate and Glucose Levels after Exposure to the Pyrolyzed By-products of SFE Formulation A
- Submit final reports on SFE Formulation A pilot studies for publication.
- Start studies on the development of an *in vitro* method for the determination of cardiac sensitization.

Prues

- There are other studies associated with the SFE Project that are to be performed to provide sufficient data with which to draw an informed conclusion. Continued technical support will be supplied to this project.
- The TMPP project will take off in a new direction; linking fast scan cyclic voltametry and microdialysis. Technical Expertise will be supplied for the development of a microelectrode containing a carbon fiber which will allow for same site sampling collection.

Naryanan, T.K., Jung

- To increase productivity in the lab.
- To continue TMPP binding studies.
- To begin culturing mouse neuroblastoma cells and human cortical neurons for experiments with TMPP.
- To continue the work with the rat liver cells of the cell model project and begin work with human liver tissue.
- To begin HPLC analysis of the neurotransmitter samples provided set up for the synthesis of DBNP.

Ritchie

Non-Lethal Measures of Toxicity: Performance Decrements (WU .1409):

- WU.1409 no longer funded.
- Completion and submittal of appropriate (1-2) journal articles.
- Procurement of nine neuroactive drugs, and implementation of initial NTAB validation experiments as outlined in WU.1605.
- Continuation of testing of pigeons on spectral and geometric discrimination problems.
- Commencement of rats studies evaluating play behavior, Porsolt Forced Swim Test, Morris Water Maze and Schedule-Induced Polydipsia tests.

Improved Methods for Evaluating Performance Deficits Induced by Brief Exposures to High Concentrations of Gases or Vapors (WU .1408):

- Procurement and developmental testing of Halon-1301 and HFC-227ea.
- Submittal of two journal articles (CFC-12 versus HFC-134a versus Halon-1211 toxicity, as measured by motor system or operant performance).

- Submittal of abstract for poster presentation at the 1996 Society of Toxicology meeting and 1996 NHEC conference.

Improved Methods to Evaluate Performance Deficits Induced by Complex Mixtures (WU.1420):

- Coordination of engineering and development of a gas delivery and analysis system for exposure of rats and pigeons to up to five fire gases.

TMPP Mechanisms of Action: Development of Neurobehavioral Molecularization Techniques (WU .1512):

- Coordination in continued microdialysis/EEG/behavioral analysis of rats during micro infusion of TMPP into selected nuclei, and start-up of cyclic voltammetry studies.
- Coordination in start-up of TMPP CNS sensitization and tissue slice studies, including assistance with > 50 rat surgeries.
- Coordination in start-up of long-term TMPP-induced developmental toxicity study.
- Coordinate completion of TMPP competitive binding studies.
- Coordinate analysis of Receptor-Select Autoradiography for TMPP binding, contracted through Kennedy-Krieger Institute.

Persian Gulf War (PGW) Simulation using Sprague-Dawley rats (U.S. Army and NMRI/TD):

- Assistance in equipment development to allow pilot study exposure (6 days) of 32 rats to combinations of chemical toxicants and environmental stressors encountered by Persian Gulf war veterans.
- Neurobehavioral testing (grip strength, endurance, learning capacity, etc.) of 50% of rats from pilot study exposure.
- Preparation for proposed 1 Jan 1996 exposures and testing of 132 rats to all combination of up to five chemical toxicants and environment stressors encountered by Persian Gulf war veterans.

Ademujohn

- To accurately and efficiently compile, log organize and analyze all incoming data from inhalation studies.
- To accurately train rodents for various testing protocols.
- To accurately train pigeons for upcoming testing protocols.
- To maintain a clean and orderly laboratory environment.

- To provide technical assistance in modified Wahmann chamber studies.
- To provide technical support in testing relative toxicity of Halon 1301 in pigeons.
- To provide technical support to operant chamber analysis of rat exposures to low concentrations of Halon 1301, R-227 EA .
- To provide technical support in streamlining operant training methods for upcoming pigeon and rodent training protocols/testing , i.e., in play behavior, swim testing and pharmacological studies.
- To procure and document pigeon maintenance pertaining to preparatory requirements for 'shaping' activities , pre-testing and testing protocols.

Martin

- Continue implementing and developing technical communication program at NMRI/TD.
- Finalize abstract booklet project.
- Prepare abstract and poster presentation for NEHC conference.
- Begin learning multimedia software for upcoming multimedia project for NMRI/TD.
- Finalize photo CD project.
- Continue to lend editorial input to Internet server updates and maintenance.

Binole, Rix

In the next quarter ADP will continue to implement those software and hardware products which increase automation and productivity. Projects scheduled for the coming quarter include:

- Continue to add to and improve our network capabilities.
- Pending guidelines from the TriService program development task force. Rework/add to internet services.
- Pending system access install SMS.
- Pending input from end users convert current Navy Supply database to SQL or FoxPro.
- Continue to provide technical support for TOXDET personnel.
- Develop support software where needed.
- Pending funding install additional CD-ROM capabilities.

Connolly

- Continue cataloging.
- Continue preparing cards for the manual card catalog.
- Continue training program.
- Set up journal routing slips to ensure better circulation of new issues of journals.

Caldwell

- Assist in preparation of an animal use protocol for Toxicity of Vapor Phase Lubricants follow-on project.
- Provide technical support to the Combustion Toxicology (F-22 and B-2) projects.

Walsh

- Complete and submit an animal use protocol for Toxicity of Vapor Phase Lubricants project.
- Provide technical support to the Metallothionein Gene Expression project.
- Provide technical support to the Combustion Toxicology/F22 project.

Geiss

Laboratory methods:
- Continue to develop molecular methods for use in our lab.
- Continue to assist in training scientists in molecular methods.

Group Administrator:
- Continue to perform assigned tasks.
- Assist in the coordination of a chemical inventory and hazardous materials processing.
- Develop a group mission statement and list of research objectives.

TCE and EI:
- Continue to develop probes for use in hybridization experiments.

McDougal

- Continue pharmacokinetic studies for 3 chemicals in hairless guinea pigs.

- Continue development of mathematical models for the diffusion cells.
- Measure diffusion of MACS across human skin.
- Begin estimating permeability constants for MACS components.

Grabau

- Continue support of the active research projects and Program Development efforts listed in section III, to include any additional projects that are identified.
- Continue development of the Tri-Service Toxicology Scientific Visualization and Analysis Program.

Narayanan, L.

- Measure neurotransmitters and their major metabolite levels in control and Quadricyclane exposed rats using HPLC.
- Complete and resubmit the "Induction of liver phospholipase-C activity and diacylglycerol by the peroxisome proliferator, perfluorodecanoic acid" project.
- Measure neurotoxic and acetylcholine esterase enzyme levels in different regions of rat brain.